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(54) Title: **COMPOSITIONS AND METHODS FOR ENHANCED ABSORPTION OF HYDROPHILIC THERAPEUTIC AGENTS**

(57) Abstract: The present invention relates to pharmaceutical compositions, pharmaceutical systems, and methods for enhanced absorption of hydrophilic therapeutic agents. Compositions and systems of the present invention include an absorption enhancing carrier, where the carrier is formed from a combination of at least two surfactants, at least one of which is hydrophilic. A hydrophilic therapeutic agent can be incorporated into the composition, or can be co-administered with the composition as part of a pharmaceutical system. The invention also provides methods of treatment with hydrophilic therapeutic agents using these compositions and systems.

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**COMPOSITIONS AND METHODS FOR
ENHANCED ABSORPTION OF HYDROPHILIC
THERAPEUTIC AGENTS**

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FIELD OF THE INVENTION

The present invention relates to drug, nutrient and diagnostic agent delivery systems, and in particular to pharmaceutical systems and methods for the improved delivery and enhanced absorption of hydrophilic therapeutic agents.

BACKGROUND

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Hydrophilic therapeutic agents present difficult problems in formulation. While these therapeutic agents are readily soluble in water, and are easily dissolved in the gastrointestinal environment, simple dissolution is not sufficient to provide efficient bioabsorption of the therapeutic agent. Barriers to absorption are presented by the mucous layer, the intestinal epithelial cell membrane, and the junctional structure such as tight junctions between the epithelial cells. Due to the presence of the negatively charged mucosal layer, significant electrostatic binding or repulsion of charged molecules can be encountered. The epithelial cell membranes are composed of phospholipid bilayers in which proteins are embedded via the hydrophobic segments. These bilayers at the apical and/or basolateral cell surface represent very strong barriers for transport of hydrophilic substances, including peptides and proteins. Frequently, hydrophilic therapeutic agents are also subject to enzymatic attack and are degraded before they can be presented to the absorption site.

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Some hydrophilic drugs such as acyclovir, foscarnet, tiludronate, pamidronate, alendronate, acarbose, cromolyn sodium, aminoglycoside and cephalosporin antibiotics are poorly absorbed from the gastro-intestinal tract, due to their low octanol-water partition coefficient, charge, and/or size.

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Large water-soluble polymers, such as peptides, proteins, genetic material, vaccines and oligonucleotides, are not well absorbed from the intestine, primarily due to their low membrane permeability and enzymatic inactivation. The mammalian body possesses several efficient mechanisms to restrict the entry of macromolecules. These mechanisms include the presence of significant levels of enzymatic activity at various locations prior to entry into systemic circulation.

1 Thus, numerous barriers to absorption of hydrophilic therapeutic agents are present, and these barriers inhibit the effective absorption both of small hydrophilic therapeutic agents, such as conventional non-peptidic drugs, and of macromolecular hydrophilic therapeutic agents, such as proteins, peptides, vaccines and the like.

5 Much effort has been expended to develop methods of overcoming these absorption barriers. For example, the enzymatic barrier can be attacked by administering enzyme inhibitors to prevent or at least lessen the extent of presystemic degradation in the gastrointestinal tract (see, e.g., Bernkop-Schnurch, "The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and 10 proteins", *Journal of Controlled Release*, 52, 1-16 (1998)). Other efforts have focused on, for example, the use of absorption promoters to enhance epithelial permeability (e.g., LeCluyse and Sutton, "In vitro models for selection of development candidates. Permeability studies to define mechanisms of absorption enhancement", *Advanced Drug Delivery Reviews*, 23, 163-183 (1997)). However, the effectiveness of absorption 15 enhancers such as permeability enhancers or enzyme inhibitors depends upon the ability of a pharmaceutical carrier to effectively present the absorption enhancers and the hydrophilic therapeutic agent to the absorption site, and prior efforts have not provided carriers which can do so efficiently. Moreover, maintaining effective carrier concentrations at the epithelium is not easily controlled in vivo. Too little carrier, or 20 carrier concentrations only briefly maintained, may be ineffective. Too much carrier, or carrier concentrations maintained for too long, may result in compromised safety.

25 Frequently, carrier compositions for hydrophilic therapeutic agents include or are based on triglycerides. For example, U.S. Patent Nos. 5,444,041, 5,646,109 and 5,633,226 to Owen et al. are directed to water-in-oil ("w/o") microemulsions for delivering water-soluble biological actives, such as proteins or peptides. The water-in-oil microemulsions convert into oil-in-water ("o/w") emulsions upon ingestion. The active agent is initially stored in the internal water phase of the w/o microemulsion, and is released when the composition converts to an o/w emulsion upon mixing with bodily fluids. Other oil-based or oil-containing formulations are taught in, for example, U.S. Patent No. 5,120,710 to 30 Liedtke, U.S. Patent No. 5,656,289 to Cho et al. These triglyceride-containing formulations, however, suffer from several disadvantages.

1 U.S. Patent No. 5,206,219 to Desai, for example, teaches compositions having a
particle size of 5 to 50 microns. Typically, emulsions formed from triglyceride-containing
compositions contain colloidal oil particles which are relatively large, ranging from
several hundred nanometers to several microns in diameter, in a broad particle size
distribution. Since the particle sizes are on the order of or greater than the wavelength
5 range of visible light, such emulsions, when prepared in an emulsion dosage form, are
visibly "cloudy" or "milky" to the naked eye. Emulsions are thermodynamically unstable,
and colloidal emulsion particles will spontaneously agglomerate, eventually leading to
complete phase separation. The tendency to agglomerate and phase separate presents
10 problems of storage and handling, and increases the likelihood that pharmaceutical
emulsions initially properly prepared will be in a less optimal, less effective, and poorly-
characterized state upon ultimate administration to a patient. Uncharacterized degradation
is particularly disadvantageous, since increased particle size slows the rate of transport of
15 the colloidal particle and digestion of the oil component, and hence the rate and extent of
absorption of the therapeutic agent. These problems lead to poorly-characterized and
potentially harmful changes in the effective dosage received by the patient, and/or the rate
of drug uptake. Moreover, changes in colloidal emulsion particle size are also believed to
render absorption more sensitive to and dependent upon conditions in the gastrointestinal
tract, such as pH, enzyme activity, bile components, and stomach contents. Such
20 uncertainty in the rate and extent of ultimate absorption of the therapeutic agent severely
compromises the medical professional's ability to safely administer therapeutically
effective dosages. In addition, when such compositions are administered parenterally, the
presence of large particles can block blood capillaries, further compromising patient
safety.

25 U.S. Patent No. 5,626,869 to Nyqvist et al. discloses compositions that would
likely produce discrete lipid particles of relatively large size *in vivo*. Such particles suffer
from the disadvantages of large size and low diffusivity, and are unable to effectively
present any absorption enhancing components to the site of absorption.

30 A further disadvantage of conventional triglyceride-containing compositions is the
dependence of therapeutic agent absorption on the rate and extent of lipolysis. Ultimately
the triglyceride must be digested and the therapeutic agent must be released in order to be
absorbed through the intestinal mucosa. The triglyceride carrier is emulsified by bile salts

1 and hydrolyzed, primarily by pancreatic lipase. The rate and extent of lipolysis, however,
are dependent upon several factors that are difficult to adequately control. For example,
the amount and rate of bile salt secretion affect the lipolysis of the triglycerides, and the
bile salt secretion can vary with stomach contents, with metabolic abnormalities, and with
5 functional changes of the liver, bile ducts, gall bladder and intestine. Lipase availability in
patients with decreased pancreatic secretory function, such as cystic fibrosis or chronic
pancreatitis, may be undesirably low, resulting in a slow and incomplete triglyceride
lipolysis. The activity of lipase is pH dependent, with deactivation occurring at about pH
3, so that the lipolysis rate will vary with stomach contents, and may be insufficient in
10 patients with gastric acid hyper-secretion. Moreover, certain surfactants commonly used
in the preparation of pharmaceutical emulsions, such as polyethoxylated castor oils, may
themselves act as inhibitors of lipolysis.

Other carrier formulations avoid the use of triglycerides, but still suffer
disadvantages. For example, U.S. Patent No. 5,653,987 to Modi et al. is directed to
15 pharmaceutical formulations for oral or nasal delivery of proteinaceous pharmaceutical
agents using small amounts of particular surfactants and a protease inhibitor in an aqueous
medium as absorption enhancers. However, in the gastrointestinal tract, where the volume
of liquids is large and motility is great, polar drugs and the protease inhibitor are diluted
even further upon administration, thus negating any potential benefits, since the
20 composition is unable to deliver meaningful amounts of the absorption enhancers and
pharmaceutical agents to the absorption site.

Thus, there is a need for pharmaceutical compositions that overcome the
limitations of conventional formulations, to provide effective delivery of absorption
enhancers and enhanced absorption of hydrophilic therapeutic agents.
25

SUMMARY OF THE INVENTION

The present invention provides triglyceride-free pharmaceutical systems for
enhanced bioabsorption of hydrophilic therapeutic agents. It has been surprisingly found
that pharmaceutical compositions having absorption enhancing properties can be provided
by using a combination of surfactants in amounts such that when the pharmaceutical
30 composition is mixed with an aqueous diluent, an aqueous dispersion having a very small
average particle size is formed. Such compositions can be co-administered with a
hydrophilic therapeutic agent to increase the rate and/or extent of bioabsorption of the

1 hydrophilic therapeutic agent, or can be provided with a hydrophilic therapeutic agent in
the preconcentrate composition or in a diluent solution.

In one embodiment, the present invention relates to triglyceride-free pharmaceutical systems having a dosage form of an absorption enhancing composition
5 comprising at least two surfactants, at least one of which is hydrophilic, and a hydrophilic therapeutic agent. The surfactants are present in amounts such that the carrier forms an aqueous dispersion having a very small average particle size upon mixing with an aqueous diluent. The hydrophilic therapeutic agent can be solubilized, suspended, or partially solubilized and suspended, in the absorption enhancing carrier. Alternatively, the
10 hydrophilic therapeutic agent can be provided separately, for co-administration with the dosage form of the absorption enhancing composition.

In another embodiment, the present invention provides a triglyceride-free pharmaceutical system for enhanced absorption of a hydrophilic therapeutic agent, including a dosage form of an absorption enhancing composition, and a hydrophilic
15 therapeutic agent, wherein the absorption enhancing composition has at least one hydrophilic surfactant and at least one hydrophobic surfactant. The surfactants are present in amounts such that the carrier forms an aqueous dispersion having a very small average particle size upon mixing with an aqueous diluent. The hydrophilic therapeutic agent can be solubilized, suspended, or partially solubilized and suspended, in the dosage form of
20 the absorption enhancing composition, or provided in a separate dosage form.

In another embodiment, the present invention provides a method of improving the bioabsorption of a hydrophilic therapeutic agent administered to a patient. The method includes the steps of providing a dosage form of an absorption enhancing composition, providing a hydrophilic therapeutic agent, and administering the dosage form of the
25 absorption enhancing composition and the hydrophilic therapeutic agent to a patient. The method improves bioabsorption by improving the consistency of delivery of the hydrophilic therapeutic agent to the absorption site, and providing absorption enhancers at the absorption site.

These and other features of the present invention will become more fully apparent
30 from the following description and appended claims, or may be learned by the practice of the invention as set forth hereinafter.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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The present invention overcomes the problems described above characteristic of conventional formulations of hydrophilic therapeutic agents by providing unique pharmaceutical systems for enhanced absorption of hydrophilic therapeutic agents. The pharmaceutical systems include absorption-enhancing components which, when the compositions are mixed with an aqueous diluent either *in vitro* or *in vivo*, form aqueous dispersions having a very small particle size. The combination of absorption enhancing compounds at relatively high concentration, very small particle sizes upon dispersion, and the absence of triglycerides unexpectedly enhances the rate, extent and/or consistency of bioabsorption of hydrophilic therapeutic agents present in, or co-administered with, the absorption enhancing compositions.

10

The term "absorption enhancement" as used herein means an improvement in one or more of the rate of bioabsorption, the extent of bioabsorption, and the consistency of the rate and/or extent of bioabsorption. Without wishing to be bound by theory, it is believed that the absorption enhancement provided by the pharmaceutical systems of the present invention is a result of one or more of the following factors:

15

- (1) effective presentation of an absorption enhancer to the site of enhancement;
- (2) modulation of facilitated/active transport;
- (3) transcellular permeability enhancement through favorable membrane perturbations;
- (4) inhibition of efflux related transporters;
- (5) inhibition of luminal or cellular enzymatic inactivation;
- (6) paracellular transport enhancement through loosening of tight junctions;
- (7) induction of specific transporters to facilitate transport;
- (8) altered biological binding characteristics;
- (9) reduced degradation of the hydrophilic therapeutic agent;
- (10) induction of transient water channels; and/or
- (11) increased partitioning of the hydrophilic therapeutic agent by association with the absorption enhancer.

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A. Pharmaceutical Compositions and Methods

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In one embodiment, the present invention provides a triglyceride-free pharmaceutical system including an absorption enhancing composition. The absorption enhancing composition includes at least two surfactants, at least one of which is a hydrophilic surfactant. Preferably, the carrier includes at least one hydrophilic surfactant and at least one hydrophobic surfactant. The surfactants are present in amounts such that upon dilution with an aqueous diluent, either *in vitro* or *in vivo*, the carrier forms an aqueous dispersion having a small average particle size. The hydrophilic and hydrophobic surfactants are believed to function as absorption enhancers, and the hydrophilic surfactant 10 additionally assists the functionality of other absorption enhancing hydrophilic or hydrophobic surfactants.

10

1. Surfactants

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The absorption enhancing composition includes at least two surfactants, at least one of which is a hydrophilic surfactant. Preferably, the composition includes at least one hydrophilic surfactant and at least one hydrophobic surfactant. As is well known in the art, the terms "hydrophilic" and "hydrophobic" are relative terms. To function as a surfactant, a compound must necessarily include polar or charged hydrophilic moieties as well as non-polar hydrophobic (lipophilic) moieties; *i.e.*, a surfactant compound must be amphiphilic. An empirical parameter commonly used to characterize the relative 20 hydrophilicity and hydrophobicity of non-ionic amphiphilic compounds is the hydrophilic-lipophilic balance ("HLB" value). Surfactants with lower HLB values are more hydrophobic, and have greater solubility in oils, while surfactants with higher HLB values are more hydrophilic, and have greater solubility in aqueous solutions.

20

Using HLB values as a rough guide, hydrophilic surfactants are generally 25 considered to be those compounds having an HLB value greater than about 10, as well as anionic, cationic, or zwitterionic compounds for which the HLB scale is not generally applicable. Similarly, hydrophobic surfactants are compounds having an HLB value less than about 10.

30

It should be appreciated that the HLB value of a surfactant is merely a rough guide generally used to enable formulation of industrial, pharmaceutical and cosmetic emulsions. For many important surfactants, including several polyethoxylated surfactants, it has been reported that HLB values can differ by as much as about 8 HLB units,

1 depending upon the empirical method chosen to determine the HLB value (Schott, *J. Pharm. Sciences*, 79(1), 87-88 (1990)). Likewise, for certain polypropylene oxide
5 containing block copolymers (PLURONIC® surfactants, BASF Corp.), the HLB values
may not accurately reflect the true physical chemical nature of the compounds. Finally,
commercial surfactant products are generally not pure compounds, but are complex
mixtures of compounds, and the HLB value reported for a particular compound may more
accurately be characteristic of the commercial product of which the compound is a major
component. Different commercial products having the same primary surfactant
10 component can, and typically do, have different HLB values. In addition, a certain
amount of lot-to-lot variability is expected even for a single commercial surfactant
product. Keeping these inherent difficulties in mind, and using HLB values as a guide,
one skilled in the art can readily identify surfactants having suitable hydrophilicity or
hydrophobicity for use in the present invention, as described herein.

15 The hydrophilic surfactant can be any hydrophilic surfactant suitable for use in
pharmaceutical compositions. Such surfactants can be anionic, cationic, zwitterionic or
non-ionic, although non-ionic hydrophilic surfactants are presently preferred. As
discussed above, these non-ionic hydrophilic surfactants will generally have HLB values
greater than about 10. Mixtures of hydrophilic surfactants are also within the scope of the
invention.

20 Similarly, the hydrophobic surfactant can be any hydrophobic surfactant suitable
for use in pharmaceutical compositions. In general, suitable hydrophobic surfactants will
have an HLB value less than about 10. Mixtures of hydrophobic surfactants are also
within the scope of the invention.

25 The choice of specific hydrophobic and hydrophilic surfactants should be made
keeping in mind the particular hydrophilic therapeutic agent to be used in the composition,
and the range of polarity appropriate for the chosen hydrophilic therapeutic agent, as
discussed in more detail below. With these general principles in mind, a very broad range
of surfactants is suitable for use in the present invention. Such surfactants can be grouped
30 into the following general chemical classes detailed in the Tables herein. The HLB values
given in the Tables below generally represent the HLB value as reported by the
manufacturer of the corresponding commercial product. In cases where more than one
commercial product is listed, the HLB value in the Tables is the value as reported for one

1 of the commercial products, a rough average of the reported values, or a value that, in the judgment of the present inventors, is more reliable. It should be emphasized that the invention is not limited to the surfactants in the Tables, which show representative, but not exclusive, lists of available surfactants.

5 1.1. Polyethoxylated Fatty Acids

Although polyethylene glycol (PEG) itself does not function as a surfactant, a variety of PEG-fatty acid esters have useful surfactant properties. Among the PEG-fatty acid monoesters, esters of lauric acid, oleic acid, and stearic acid are especially useful. Among the surfactants of Table 1, preferred hydrophilic surfactants include PEG-8 laurate, 10 PEG-8 oleate, PEG-8 stearate, PEG-9 oleate, PEG-10 laurate, PEG-10 oleate, PEG-12 laurate, PEG-12 oleate, PEG-15 oleate, PEG-20 laurate and PEG-20 oleate. Examples of polyethoxylated fatty acid monoester surfactants commercially available are shown in Table 1.

15

Table 1: PEG-Fatty Acid Monoester Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|------------------------------|--|-----|
| PEG 4-100 monolaurate | Crodet L series (Croda) | >9 |
| PEG 4-100 monooleate | Crodet O series (Croda) | >8 |
| 20 PEG 4-100 monostearate | Crodet S series (Croda), Myrij Series (Atlas/ICI) | >6 |
| PEG 400 distearate | Cithrol 4DS series (Croda) | >10 |
| PEG 100,200,300 monolaurate | Cithrol ML series (Croda) | >10 |
| PEG 100,200,300 monooleate | Cithrol MO series (Croda) | >10 |
| PEG 400 dioleate | Cithrol 4DO series (Croda) | >10 |
| 25 PEG 400-1000 monostearate | Cithrol MS series (Croda) | >10 |
| PEG-1 stearate | Nikkol MYS-1EX (Nikko), Coster K1 (Condea) | 2 |
| PEG-2 stearate | Nikkol MYS-2 (Nikko) | 4 |
| PEG-2 oleate | Nikkol MYO-2 (Nikko) | 4.5 |
| 30 PEG-4 laurate | Mapeg® 200 ML (PPG), Kessco® PEG 200ML (Stepan), LIPOPEG 2L (LIPO Chem.) | 9.3 |
| PEG-4 oleate | Mapeg® 200 MO (PPG), Kessco® PEG200 MO (Stepan) | 8.3 |
| PEG-4 stearate | Kessco® PEG 200 MS (Stepan), Hodag 20 S (CAlgene), Nikkol MYS-4 (Nikko) | 6.5 |

| | | | |
|----|--------------------|--|------|
| 1 | PEG-5 stearate | Nikkol TMGS-5 (Nikko) | 9.5 |
| | PEG-5 oleate | Nikkol TMGO-5 (Nikko) | 9.5 |
| | PEG-6 oleate | Algon OL 60 (Auschem SpA), Kessco® PEG 300 MO (Stepan), Nikkol MYO-6 (Nikko), Emulgante A6 (Condea) | 8.5 |
| 5 | PEG-7 oleate | Algon OL 70 (Auschem SpA) | 10.4 |
| | PEG-6 laurate | Kessco® PEG300 ML (Stepan) | 11.4 |
| | PEG-7 laurate | Lauridac 7 (Condea) | 13 |
| | PEG-6 stearate | Kessco® PEG300 MS (Stepan) | 9.7 |
| | PEG-8 laurate | Mapeg® 400 ML (PPG), LIPOPEG 4DL(Lipo Chem.) | 13 |
| 10 | PEG-8 oleate | Mapeg® 400 MO (PPG), Emulgante A8 (Condea); Kessco PEG 400 MO (Stepan) | 12 |
| | PEG-8 stearate | Mapeg® 400 MS (PPG), Myrj 45 | 12 |
| | PEG-9 oleate | Emulgante A9 (Condea) | >10 |
| | PEG-9 stearate | Cremophor S9 (BASF) | >10 |
| 15 | PEG-10 laurate | Nikkol MYL-10 (Nikko), Lauridac 10 (Croda) | 13 |
| | PEG-10 oleate | Nikkol MYO-10 (Nikko) | 11 |
| | PEG-10 stearate | Nikkol MYS-10 (Nikko), Coster K100 (Condea) | 11 |
| | PEG-12 laurate | Kessco® PEG 600ML (Stepan) | 15 |
| | PEG-12 oleate | Kessco® PEG 600MO (Stepan) | 14 |
| 20 | PEG-12 ricinoleate | (CAS # 9004-97-1) | >10 |
| | PEG-12 stearate | Mapeg® 600 MS (PPG), Kessco® PEG 600MS (Stepan) | 14 |
| | PEG-15 stearate | Nikkol TMGS-15 (Nikko), Koster K15 (Condea) | 14 |
| | PEG-15 oleate | Nikkol TMGO-15 (Nikko) | 15 |
| | PEG-20 laurate | Kessco® PEG 1000 ML (Stepan) | 17 |
| 25 | PEG-20 oleate | Kessco® PEG 1000 MO (Stepan) | 15 |
| | PEG-20 stearate | Mapeg® 1000 MS (PPG), Kessco® PEG 1000 MS (Stepan), Myrj 49 | 16 |
| | PEG-25 stearate | Nikkol MYS-25 (Nikko) | 15 |
| | PEG-32 laurate | Kessco® PEG 1540 ML (Stepan) | 16 |
| 30 | PEG-32 oleate | Kessco® PEG 1540 MO (Stepan) | 17 |
| | PEG-32 stearate | Kessco® PEG 1540 MS (Stepan) | 17 |
| | PEG-30 stearate | Myrj 51 | >10 |

| | | | |
|----|------------------|---|------|
| 1 | PEG-40 laurate | Crodet L40 (Croda) | 17.9 |
| | PEG-40 oleate | Crodet O40 (Croda) | 17.4 |
| | PEG-40 stearate | Myrij 52, Emerest® 2715 (Henkel), Nikkol MYS-40 (Nikko) | >10 |
| 5 | PEG-45 stearate | Nikkol MYS-45 (Nikko) | 18 |
| | PEG-50 stearate | Myrij 53 | >10 |
| | PEG-55 stearate | Nikkol MYS-55 (Nikko) | 18 |
| | PEG-100 oleate | Crodet O-100 (Croda) | 18.8 |
| | PEG-100 stearate | Myrij 59, Arlacel 165 (ICI) | 19 |
| 10 | PEG-200 oleate | Albunol 200 MO (Taiwan Surf.) | >10 |
| | PEG-400 oleate | LACTOMUL (Henkel), Albunol 400 MO (Taiwan Surf.) | >10 |
| | PEG-600 oleate | Albunol 600 MO (Taiwan Surf.) | >10 |

1.2 PEG-Fatty Acid Diesters

15 Polyethylene glycol (PEG) fatty acid diesters are also suitable for use as surfactants in the compositions of the present invention. Among the surfactants in Table 2, preferred hydrophilic surfactants include PEG-20 dilaurate, PEG-20 dioleate, PEG-20 distearate, PEG-32 dilaurate and PEG-32 dioleate. Representative PEG-fatty acid diesters are shown in Table 2.

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Table 2: PEG-Fatty Acid Diester Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|---------------------|---|-----|
| PEG-4 dilaurate | Mapeg® 200 DL (PPG), Kessco® PEG 200 DL (Stepan), LIPOPEG 2-DL (Lipo Chem.) | 7 |
| 25 PEG-4 dioleate | Mapeg® 200 DO (PPG), | 6 |
| PEG-4 distearate | Kessco® 200 DS (Stepan) | 5 |
| PEG-6 dilaurate | Kessco® PEG 300 DL (Stepan) | 9.8 |
| PEG-6 dioleate | Kessco® PEG 300 DO (Stepan) | 7.2 |
| 30 PEG-6 distearate | Kessco® PEG 300 DS (Stepan) | 6.5 |
| PEG-8 dilaurate | Mapeg® 400 DL (PPG), Kessco® PEG 400 DL (Stepan), LIPOPEG 4 DL (Lipo Chem.) | 11 |
| PEG-8 dioleate | Mapeg® 400 DO (PPG), Kessco® PEG 400 DO (Stepan), LIPOPEG 4 DO(Lipo Chem.) | 8.8 |

| | | | |
|----|--------------------|---|------|
| 1 | PEG-8 distearate | Mapec® 400 DS (PPG), CDS 400 (Nikkol) | 11 |
| | PEG-10 dipalmitate | Polyaldo 2PKFG | >10 |
| | PEG-12 dilaurate | Kessco® PEG 600 DL (Stepan) | 11.7 |
| 5 | PEG-12 distearate | Kessco® PEG 600 DS (Stepan) | 10.7 |
| | PEG-12 dioleate | Mapec® 600 DO (PPG), Kessco® 600 DO(Stepan) | 10 |
| | PEG-20 dilaurate | Kessco® PEG 1000 DL (Stepan) | 15 |
| | PEG-20 dioleate | Kessco® PEG 1000 DO (Stepan) | 13 |
| | PEG-20 distearate | Kessco® PEG 1000 DS (Stepan) | 12 |
| 10 | PEG-32 dilaurate | Kessco® PEG 1540 DL (Stepan) | 16 |
| | PEG-32 dioleate | Kessco® PEG 1540 DO (Stepan) | 15 |
| | PEG-32 distearate | Kessco® PEG 1540 DS (Stepan) | 15 |
| | PEG-400 dioleate | Cithrol 4DO series (Croda) | >10 |
| | PEG-400 distearate | Cithrol 4DS series (Croda) | >10 |

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1.3 PEG-Fatty Acid Mono- and Di-ester Mixtures

In general, mixtures of surfactants are also useful in the present invention, including mixtures of two or more commercial surfactant products. Several PEG-fatty acid esters are marketed commercially as mixtures or mono- and diesters. Representative 20 surfactant mixtures are shown in Table 3.

Table 3: PEG-Fatty Acid Mono- and Diester Mixtures

| | COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----|----------------------------|---|-----|
| 25 | PEG 4-150 mono, dilaurate | Kessco® PEG 200-6000 mono, dilaurate (Stepan) | |
| | PEG 4-150 mono, dioleate | Kessco® PEG 200-6000 mono, dioleate (Stepan) | |
| | PEG 4-150 mono, distearate | Kessco® 200-6000 mono, distearate (Stepan) | |

1.4 Polyethylene Glycol Glycerol Fatty Acid Esters

30 Suitable PEG glycerol fatty acid esters are shown in Table 4. Among the surfactants in the Table, preferred hydrophilic surfactants are PEG-20 glyceryl laurate,

- 1 PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-20 glyceryl oleate, and PEG-30
glyceryl oleate.

5 Table 4: PEG Glycerol Fatty Acid Esters

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----------------------------|---|-----|
| PEG-20 glyceryl laurate | Tagat® L (Goldschmidt) | 16 |
| PEG-30 glyceryl laurate | Tagat® L2 (Goldschmidt) | 16 |
| PEG-15 glyceryl laurate | Glycerox L series (Croda) | 15 |
| 10 PEG-40 glyceryl laurate | Glycerox L series (Croda) | 15 |
| PEG-20 glyceryl stearate | Capmul® EMG (ABITEC), Aldo® MS-20 KFG (Lonza) | 13 |
| PEG-20 glyceryl oleate | Tagat® O (Goldschmidt) | >10 |
| PEG-30 glyceryl oleate | Tagat® O2 (Goldschmidt) | >10 |

15 1.5. Alcohol - Oil Transesterification Products

A large number of surfactants of different degrees of hydrophobicity or hydrophilicity can be prepared by reaction of alcohols or polyalcohols with a variety of natural and/or hydrogenated oils. Most commonly, the oils used are castor oil or hydrogenated castor oil, or an edible vegetable oil such as corn oil, olive oil, peanut oil, palm kernel oil, apricot kernel oil, or almond oil. Preferred alcohols include glycerol, propylene glycol, ethylene glycol, polyethylene glycol, maltol, sorbitol, and pentaerythritol. Among these alcohol-oil transesterified surfactants, preferred hydrophilic surfactants are PEG-35 castor oil (Incrocas-35), PEG-40 hydrogenated castor oil (Cremophor RH 40), PEG-25 trioleate (TAGAT® TO), PEG-60 corn glycerides (Crovol M70), PEG-60 almond oil (Crovol A70), PEG-40 palm kernel oil (Crovol PK70), PEG-50 castor oil (Emalex C-50), PEG-50 hydrogenated castor oil (Emalex HC-50), PEG-8 caprylic/capric glycerides (Labrasol), and PEG-6 caprylic/capric glycerides (Softigen 767). Preferred hydrophobic surfactants in this class include PEG-5 hydrogenated castor oil, PEG-7 hydrogenated castor oil, PEG-9 hydrogenated castor oil, PEG-6 corn oil (Labrafil® M 2125 CS), PEG-6 almond oil (Labrafil® M 1966 CS), PEG-6 apricot kernel oil (Labrafil® M 1944 CS), PEG-6 olive oil (Labrafil® M 1980 CS), PEG-6 peanut oil (Labrafil® M 1969 CS), PEG-6 hydrogenated palm kernel oil (Labrafil® M 2130 BS),

1 PEG-6 palm kernel oil (Labrafil® M 2130 CS), PEG-6 triolein (Labrafil® M 2735 CS),
 PEG-8 corn oil (Labrafil® WL 2609 BS), PEG-20 corn glycerides (Crovil M40), and
 PEG-20 almond glycerides (Crovil A40). The latter two surfactants are reported to have
 5 HLB values of 10, which is generally considered to be the approximate border line
 between hydrophilic and hydrophobic surfactants. For purposes of the present invention,
 these two surfactants are considered to be hydrophobic. Representative surfactants of this
 class suitable for use in the present invention are shown in Table 5.

10 Table 5: Transesterification Products of Oils and Alcohols

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|-----------------------------------|--|-----|
| PEG-3 castor oil | Nikkol CO-3 (Nikko) | 3 |
| PEG-5, 9, and 16 castor oil | ACCONON CA series (ABITEC) | 6-7 |
| PEG-20 castor oil | Emalex C-20 (Nihon Emulsion), Nikkol CO-20 TX (Nikko) | 11 |
| 15 PEG-23 castor oil | Emulgante EL23 | >10 |
| PEG-30 castor oil | Emalex C-30 (Nihon Emulsion), Alkamuls® EL 620 (Rhone-Poulenc), Incrocas 30 (Croda) | 11 |
| PEG-35 castor oil | Cremophor EL and EL-P (BASF), Emulphor EL, Incrocas-35 (Croda), Emulgin RO 35 (Henkel) | |
| 20 PEG-38 castor oil | Emulgante EL 65 (Condea) | |
| PEG-40 castor oil | Emalex C-40 (Nihon Emulsion), Alkamuls® EL 719 (Rhone-Poulenc) | 13 |
| PEG-50 castor oil | Emalex C-50 (Nihon Emulsion) | 14 |
| PEG-56 castor oil | Eurnulgin® PRT 56 (Pulcra SA) | >10 |
| PEG-60 castor oil | Nikkol CO-60TX (Nikko) | 14 |
| 25 PEG-100 castor oil | Thornley | >10 |
| PEG-200 castor oil | Eurnulgin® PRT 200 (Pulcra SA) | >10 |
| PEG-5 hydrogenated castor oil | Nikkol HCO-5 (Nikko) | 6 |
| PEG-7 hydrogenated castor oil | Simusol® 989 (Seppic), Cremophor WO7 (BASF) | 6 |
| PEG-10 hydrogenated castor oil | Nikkol HCO-10 (Nikko) | 6.5 |
| 30 PEG-20 hydrogenated castor oil | Nikkol HCO-20 (Nikko) | 11 |
| PEG-25 hydrogenated castor oil | Simulsol® 1292 (Seppic), Cerex ELS 250 (Auschem SpA) | 11 |
| PEG-30 hydrogenated castor oil | Nikkol HCO-30 (Nikko) | 11 |

| | | | |
|----|--|--|-----|
| 1 | PEG-40 hydrogenated castor oil | Cremophor RH 40 (BASF), Croduret (Croda), Emulgin HRE 40 (Henkel) | 13 |
| | PEG-45 hydrogenated castor oil | Cerex ELS 450 (Auschem Spa) | 14 |
| | PEG-50 hydrogenated castor oil | Emalex HC-50 (Nihon Emulsion) | 14 |
| 5 | PEG-60 hydrogenated castor oil | Nikkol HCO-60 (Nikko); Cremophor RH 60 (BASF) | 15 |
| | PEG-80 hydrogenated castor oil | Nikkol HCO-80 (Nikko) | 15 |
| | PEG-100 hydrogenated castor oil | Nikkol HCO -100 (Nikko) | 17 |
| | PEG-6 corn oil | Labrafil® M 2125 CS (Gattefosse) | 4 |
| 10 | PEG-6 almond oil | Labrafil® M 1966 CS (Gattefosse) | 4 |
| | PEG-6 apricot kernel oil | Labrafil® M 1944 CS (Gattefosse) | 4 |
| | PEG-6 olive oil | Labrafil® M 1980 CS (Gattefosse) | 4 |
| | PEG-6 peanut oil | Labrafil® M 1969 CS (Gattefosse) | 4 |
| | PEG-6 hydrogenated palm kernel oil | Labrafil® M 2130 BS (Gattefosse) | 4 |
| 15 | PEG-6 palm kernel oil | Labrafil® M 2130 CS (Gattefosse) | 4 |
| | PEG-6 triolein | Labrafil® M 2735 CS (Gattefosse) | 4 |
| | PEG-8 corn oil | Labrafil® WL 2609 BS (Gattefosse) | 6-7 |
| | PEG-20 corn glycerides | Crovol M40 (Croda) | 10 |
| 20 | PEG-20 almond glycerides | Crovol A40 (Croda) | 10 |
| | PEG-25 trioleate | TAGAT® TO (Goldschmidt) | 11 |
| | PEG-40 palm kernel oil | Crovol PK-70 | >10 |
| | PEG-60 corn glycerides | Crovol M70(Croda) | 15 |
| | PEG-60 almond glycerides | Crovol A70 (Croda) | 15 |
| 25 | PEG-4 caprylic/capric triglyceride | Labrafac® Hydro (Gattefosse), Labrafac® Hydro (Gattefosse) | 4-5 |
| | PEG-8 caprylic/capric glycerides | Labrasol (Gattefosse),Labrafac CM 10 (Gattefosse) | >10 |
| | PEG-6 caprylic/capric glycerides | SOFTIGEN® 767 (Hüls), Glycerox 767 (Croda) | 19 |
| | Lauroyl macrogol-32 glyceride | GELUCIRE 44/14 (Gattefosse) | 14 |
| 30 | Stearoyl macrogol glyceride | GELUCIRE 50/13 (Gattefosse) | 13 |
| | Mono, di, tri, tetra esters of vegetable oils and sorbitol | SorbitoGlyceride (Gattefosse) | <10 |
| | Pentaerythrityl tetraisostearate | Crodamol PTIS (Croda) | <10 |

| | | | |
|---|--|--|-----|
| 1 | Pentaerythrityl distearate | Albunol DS (Taiwan Surf.) | <10 |
| | Pentaerythrityl tetraoleate | Liponate PO-4 (Lipo Chem.) | <10 |
| | Pentaerythrityl tetrastearate | Liponate PS-4 (Lipo Chem.) | <10 |
| 5 | Pentaerythrityl tetracaprylate/tetracaprate | Liponate PE-810 (Lipo Chem.), Crodamol PTC (Croda) | <10 |
| | Pentaerythrityl tetraoctanoate | Nikkol Pentarate 408 (Nikko) | |

Also included as oils in this category of surfactants are oil-soluble vitamins, such as vitamins A, D, E, K, etc. Thus, derivatives of these vitamins, such as tocopheryl PEG-1000 succinate (TPGS, available from Eastman), are also suitable surfactants.

1.6. Polyglycerized Fatty Acids

Polyglycerol esters of fatty acids are also suitable surfactants for the present invention. Among the polyglyceryl fatty acid esters, preferred hydrophobic surfactants include polyglyceryl oleate (Plurol Oleique), polyglyceryl-2 dioleate (Nikkol DGDO), and polyglyceryl-10 trioleate. Preferred hydrophilic surfactants include polyglyceryl-10 laurate (Nikkol Decaglyn 1-L), polyglyceryl-10 oleate (Nikkol Decaglyn 1-O), and polyglyceryl-10 mono, dioleate (Caprol® PEG 860). Polyglyceryl polyricinoleates (Polymuls) are also preferred hydrophilic and hydrophobic surfactants. Examples of suitable polyglyceryl esters are shown in Table 6.

Table 6: Polyglycerized Fatty Acids

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----------------------------|---|-----|
| Polyglyceryl-2 stearate | Nikkol DGMS (Nikko) | 5-7 |
| 25 Polyglyceryl-2 oleate | Nikkol DGMO (Nikko) | 5-7 |
| Polyglyceryl-2 isostearate | Nikkol DGMIS (Nikko) | 5-7 |
| Polyglyceryl-3 oleate | Caprol® 3GO (ABITEC), Drewpol 3-1-O (Stepan) | 6.5 |
| Polyglyceryl-4 oleate | Nikkol Tetraglyn 1-O (Nikko) | 5-7 |
| 30 Polyglyceryl-4 stearate | Nikkol Tetraglyn 1-S (Nikko) | 5-6 |
| Polyglyceryl-6 oleate | Drewpol 6-1-O (Stepan), Nikkol Hexaglyn 1-O (Nikko) | 9 |
| Polyglyceryl-10 laurate | Nikkol Decaglyn 1-L (Nikko) | 15 |
| Polyglyceryl-10 oleate | Nikkol Decaglyn 1-O (Nikko) | 14 |

| | | | |
|----|---------------------------------|---|------|
| 1 | Polyglyceryl-10 stearate | Nikkol Decaglyn 1-S (Nikko) | 12 |
| | Polyglyceryl-6 ricinoleate | Nikkol Hexaglyn PR-15 (Nikko) | >8 |
| | Polyglyceryl-10 linoleate | Nikkol Decaglyn 1-LN (Nikko) | 12 |
| 5 | Polyglyceryl-6 pentaoleate | Nikkol Hexaglyn 5-O (Nikko) | <10 |
| | Polyglyceryl-3 dioleate | Cremophor GO32 (BASF) | <10 |
| | Polyglyceryl-3 distearate | Cremophor GS32 (BASF) | <10 |
| | Polyglyceryl-4 pentaoleate | Nikkol Tetraglyn 5-O (Nikko) | <10 |
| | Polyglyceryl-6 dioleate | Caprol® 6G20 (ABITEC); Hodag PGO-62 (Calgene), PLUROL OLEIQUE CC 497 (Gattefosse) | 8.5 |
| 10 | Polyglyceryl-2 dioleate | Nikkol DGDO (Nikko) | 7 |
| | Polyglyceryl-10 trioleate | Nikkol Decaglyn 3-O (Nikko) | 7 |
| | Polyglyceryl-10 pentaoleate | Nikkol Decaglyn 5-O (Nikko) | 3.5 |
| | Polyglyceryl-10 septooleate | Nikkol Decaglyn 7-O (Nikko) | 3 |
| 15 | Polyglyceryl-10 tetraoleate | Caprol® 10G40 (ABITEC); Hodag PGO-62 (CALGENE), Drewpol 10-4-O (Stepan) | 6.2 |
| | Polyglyceryl-10 decaisostearate | Nikkol Decaglyn 10-IS (Nikko) | <10 |
| | Polyglyceryl-10I decaoleate | Drewpol 10-10-O (Stepan), Caprol 10G10O (ABITEC), Nikkol Decaglyn 10-O | 3.5 |
| | Polyglyceryl-10 mono, dioleate | Caprol® PGE 860 (ABITEC) | 11 |
| 20 | Polyglyceryl polyricinoleate | Polymuls (Henkel) | 3-20 |

1.7. Propylene Glycol Fatty Acid Esters

Esters of propylene glycol and fatty acids are suitable surfactants for use in the present invention. In this surfactant class, preferred hydrophobic surfactants include 25 propylene glycol monolaurate (Lauroglycol FCC), propylene glycol ricinoleate (Propymuls), propylene glycol monooleate (Myverol P-O6), propylene glycol dicaprylate/dicaprate (Captex® 200), and propylene glycol dioctanoate (Captex® 800). Examples of surfactants of this class are given in Table 7.

1

Table 7: Propylene Glycol Fatty Acid Esters

| | COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|--|--|--|-----|
| 5 | Propylene glycol monocaprylate | Capryol 90 (Gattefosse), Nikkol Sefsol 218 (Nikko) | <10 |
| Propylene glycol monolaurate | Lauroglycol 90 (Gattefosse), Lauroglycol FCC (Gattefosse) | <10 | |
| Propylene glycol oleate | Lutrol OP2000 (BASF) | <10 | |
| Propylene glycol myristate | Mirypol | <10 | |
| Propylene glycol monostearate | ADM PGME-03 (ADM), LIPO PGMS (Lipo Chem.), Aldo® PGHMS (Lonza) | 3-4 | |
| 10 | Propylene glycol hydroxy stearate | | <10 |
| Propylene glycol ricinoleate | PROPYMULS (Henkel) | <10 | |
| Propylene glycol isostearate | | <10 | |
| Propylene glycol monooleate | Myverol P-O6 (Eastman) | <10 | |
| Propylene glycol dicaprylate/dicaprate | Captex® 200 (ABITEC), Miglyol® 840 (Hüls), Neobee® M-20 (Stepan) | >6 | |
| 15 | Propylene glycol dioctanoate | Captex® 800 (ABITEC) | >6 |
| Propylene glycol caprylate/caprate | LABRAFAC PG (Gattefosse) | >6 | |
| Propylene glycol dilaurate | | >6 | |
| Propylene glycol distearate | Kessco® PGDS (Stepan) | >6 | |
| 20 | Propylene glycol dicaprylate | Nikkol Sefsol 228 (Nikko) | >6 |
| Propylene glycol dicaprate | Nikkol PDD (Nikko) | >6 | |

Table 7 includes both mono- and diesters of propylene glycol, and both may be used in one embodiment of the pharmaceutical systems of the present invention. In another embodiment, the absorption enhancing composition is free of both triglycerides and propylene glycol diesters.

1.8. Mixtures of Propylene Glycol Esters - Glycerol Esters

In general, mixtures of surfactants are also suitable for use in the present invention. In particular, mixtures of propylene glycol fatty acid esters and glycerol fatty acid esters are suitable and are commercially available. One preferred mixture is composed of the oleic acid esters of propylene glycol and glycerol (Arlacel 186). Examples of these surfactants are shown in Table 8.

Table 8: Glycerol/Propylene Glycol Fatty Acid Esters

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----------|-------------------------------|-----|
| Oleic | ATMOS 300, ARLACEL 186 (ICI) | 3-4 |
| Stearic | ATMOS 150 | 3-4 |

1.9. Mono- and Diglycerides

A particularly important class of surfactants is the class of mono- and diglycerides. These surfactants are generally hydrophobic. Preferred hydrophobic surfactants in this class of compounds include glyceryl monooleate (Peceol), glyceryl ricinoleate, glyceryl laurate, glyceryl dilaurate (Capmul® GDL), glyceryl dioleate (Capmul® GDO), glyceryl mono/dioleate (Capmul® GMO-K), glyceryl caprylate/caprate (Capmul® MCM), caprylic acid mono/diglycerides (Imwitor® 988), and mono- and diacetylated monoglycerides (Myvacet® 9-45). Examples of these surfactants are given in Table 9.

Table 9: Mono- and Diglyceride Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|-------------------------------|---|-----|
| Monopalmitolein (C16:1) | (Larodan) | <10 |
| Monolauidin (C18:1) | (Larodan) | <10 |
| Monocaproin (C6) | (Larodan) | <10 |
| Monocaprylin | (Larodan) | <10 |
| Monocaprin | (Larodan) | <10 |
| Monolaurin | (Larodan) | <10 |
| Glyceryl monomyristate (C14) | Nikkol MGM (Nikko) | 3-4 |
| Glyceryl monooleate (C18:1) | PECEOL (Gattefosse), Hodag GMO-D, Nikkol MGO (Nikko) | 3-4 |
| Glyceryl monooleate | RYLO series (Danisco), DIMODAN series (Danisco), EMULDAN (Danisco), ALDO® MO FG (Lonza), Kessco GMO (Stepan), MONOMULS® series (Henkel), TEGIN O, DREWMLULSE GMO (Stepan), Atlas G-695 (ICI), GMOrphic 80 (Eastman), ADM DMG-40, 70, and 100 (ADM), Myverol (Eastman) | 3-4 |
| Glycerol monooleate/linoleate | OLICINE (Gattefosse) | 3-4 |

| | | | |
|----|---|--|-------|
| 1 | Glycerol monolinoleate | Maisine (Gattefosse), MYVEROL 18-92, Myverol 18-06 (Eastman) | 3-4 |
| | Glyceryl ricinoleate | Softigen® 701 (Hüls), HODAG GMR-D (Calgene), ALDO® MR (Lonza) | 6 |
| | Glyceryl monolaurate | ALDO® MLD (Lonza), Hodag GML (Calgene) | 6.8 |
| 5 | Glycerol monopalmitate | Emalex GMS-P (Nihon) | 4 |
| | Glycerol monostearate | Capmul® GMS (ABITEC), Myvaplex (Eastman), IMWITOR® 191 (Hüls), CUTINA GMS, Aldo® MS (Lonza), Nikkol MGS series (Nikko) | 5-9 |
| | Glyceryl mono-,dioleate | Capmul® GMO-K (ABITEC) | <10 |
| | Glyceryl palmitic/stearic | CUTINA MD-A, ESTAGEL-G18 | <10 |
| 10 | Glyceryl acetate | Lamegin® EE (Grünaau GmbH) | <10 |
| | Glyceryl laurate | Imwitor® 312 (Hüls), Monomuls® 90-45 (Grünaau GmbH), Aldo® MLD (Lonza) | 4 |
| | Glyceryl citrate/lactate/oleate/ linoleate | Imwitor® 375 (Hüls) | <10 |
| 15 | Glyceryl caprylate | Imwitor® 308 (Hüls), Capmul® MCMC8 (ABITEC) | 5-6 |
| | Glyceryl caprylate/caprate | Capmul® MCM (ABITEC) | 5-6 |
| | Caprylic acid mono,diglycerides | Imwitor® 988 (Hüls) | 5-6 |
| | Caprylic/capric glycerides | Imwitor® 742 (Hüls) | <10 |
| | Mono- and diacylated monoglycerides | Myvacet® 9-45, Myvacet® 9-40, Myvacet® 9-08 (Eastman), Lamegin® (Grünaau) | 3.8-4 |
| 20 | Glyceryl monostearate | Aldo® MS, Arlaclel 129 (ICI), LIPO GMS (Lipo Chem.), Imwitor® 191 (Hüls), Myvaplex (Eastman) | 4.4 |
| | Lactic acid esters of mono,diglycerides | LAMEGIN GLP (Henkel) | <10 |
| | Dicaproin (C6) | (Larodan) | <10 |
| 25 | Dicaprin (C10) | (Larodan) | <10 |
| | Dioctanoin (C8) | (Larodan) | <10 |
| | Dimyristin (C14) | (Larodan) | <10 |
| | Dipalmitin (C16) | (Larodan) | <10 |
| | Distearin | (Larodan) | <10 |
| 30 | Glyceryl dilaurate (C12) | Capmul® GDL (ABITEC) | 3-4 |
| | Glyceryl dioleate | Capmul® GDO (ABITEC) | 3-4 |
| | Glycerol esters of fatty acids | GELUCIRE 39/01 (Gattefosse), GELUCIRE 43/01 (Gattefosse) | 1 |

| | | | |
|---|-----------------------------|-----------------------------|-----|
| 1 | | GELUCIRE 37/06 (Gattefosse) | 6 |
| | Dipalmitolein (C16:1) | (Larodan) | <10 |
| | 1,2 and 1,3-diolein (C18:1) | (Larodan) | <10 |
| 5 | Dielaidin (C18:1) | (Larodan) | <10 |
| | Dilinolein (C18:2) | (Larodan) | <10 |

1.10. Sterol and Sterol Derivatives

10 Sterols and derivatives of sterols are suitable surfactants for use in the present invention. These surfactants can be hydrophilic or hydrophobic. Preferred derivatives include the polyethylene glycol derivatives. A preferred hydrophobic surfactant in this class is cholesterol. A preferred hydrophilic surfactant in this class is PEG-24 cholesterol ether (Solulan C-24). Examples of surfactants of this class are shown in Table 10.

15

Table 10: Sterol and Sterol Derivative Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|-------------------------------------|-------------------------------|-----|
| Cholesterol, sitosterol, lanosterol | | <10 |
| PEG-24 cholesterol ether | Solulan C-24 (Amerchol) | >10 |
| 20 PEG-30 cholestanol | Nikkol DHC (Nikko) | >10 |
| Phytosterol | GENEROL series (Henkel) | <10 |
| PEG-25 phytosterol | Nikkol BPSH-25 (Nikko) | >10 |
| PEG-5 soya sterol | Nikkol BPS-5 (Nikko) | <10 |
| PEG-10 soya sterol | Nikkol BPS-10 (Nikko) | <10 |
| 25 PEG-20 soya sterol | Nikkol BPS-20 (Nikko) | <10 |
| PEG-30 soya sterol | Nikkol BPS-30 (Nikko) | >10 |

1.11. Polyethylene Glycol Sorbitan Fatty Acid Esters

30 A variety of PEG-sorbitan fatty acid esters are available and are suitable for use as surfactants in the present invention. In general, these surfactants are hydrophilic, although several hydrophobic surfactants of this class can be used. Among the PEG-sorbitan fatty acid esters, preferred hydrophilic surfactants include PEG-20 sorbitan monolaurate

- 1 (Tween-20), PEG-20 sorbitan monopalmitate (Tween-40), PEG-20 sorbitan monostearate
 (Tween-60), and PEG-20 sorbitan monooleate (Tween-80). Examples of these surfactants
 are shown in Table 11.

5

Table 11: PEG-Sorbitan Fatty Acid Esters

| | COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----|---------------------------------|--|-----|
| | PEG-10 sorbitan laurate | Liposorb L-10 (Lipo Chem.) | >10 |
| | PEG-20 sorbitan monolaurate | Tween-20 (Atlas/ICI), Crillet 1 (Croda), DACOL MLS 20 (Condea) | 17 |
| 10 | PEG-4 sorbitan monolaurate | Tween-21 (Atlas/ICI), Crillet 11 (Croda) | 13 |
| | PEG-80 sorbitan monolaurate | Hodag PSML-80 (Calgene); T-Maz 28 | >10 |
| | PEG-6 sorbitan monolaurate | Nikkol GL-1 (Nikko) | 16 |
| | PEG-20 sorbitan monopalmitate | Tween-40 (Atlas/ICI), Crillet 2 (Croda) | 16 |
| | PEG-20 sorbitan monostearate | Tween-60 (Atlas/ICI), Crillet 3 (Croda) | 15 |
| 15 | PEG-4 sorbitan monostearate | Tween-61 (Atlas/ICI), Crillet 31 (Croda) | 9.6 |
| | PEG-8 sorbitan monostearate | DACOL MSS (Condea) | >10 |
| | PEG-6 sorbitan monostearate | Nikkol TS106 (Nikko) | 11 |
| | PEG-20 sorbitan tristearate | Tween-65 (Atlas/ICI), Crillet 35 (Croda) | 11 |
| | PEG-6 sorbitan tetraстearate | Nikkol GS-6 (Nikko) | 3 |
| 20 | PEG-60 sorbitan tetraстearate | Nikkol GS-460 (Nikko) | 13 |
| | PEG-5 sorbitan monooleate | Tween-81 (Atlas/ICI), Crillet 41 (Croda) | 10 |
| | PEG-6 sorbitan monooleate | Nikkol TO-106 (Nikko) | 10 |
| | PEG-20 sorbitan monooleate | Tween-80 (Atlas/ICI), Crillet 4 (Croda) | 15 |
| 25 | PEG-40 sorbitan oleate | Emalex ET 8040 (Nihon Emulsion) | 18 |
| | PEG-20 sorbitan trioleate | Tween-85 (Atlas/ICI), Crillet 45 (Croda) | 11 |
| | PEG-6 sorbitan tetraoleate | Nikkol GO-4 (Nikko) | 8.5 |
| | PEG-30 sorbitan tetraoleate | Nikkol GO-430 (Nikko) | 12 |
| | PEG-40 sorbitan tetraoleate | Nikkol GO-440 (Nikko) | 13 |
| 30 | PEG-20 sorbitan monoisostearate | Tween-120 (Atlas/ICI), Crillet 6 (Croda) | >10 |
| | PEG sorbitol hexaoleate | Atlas G-1086 (ICI) | 10 |
| | PEG-6 sorbitol hexastearate | Nikkol GS-6 (Nikko) | 3 |

1 1.12. Polyethylene Glycol Alkyl Ethers

Ethers of polyethylene glycol and alkyl alcohols are suitable surfactants for use in the present invention. Preferred hydrophobic ethers include PEG-3 oleyl ether (Volpo 3) and PEG-4 lauryl ether (Brij 30). Examples of these surfactants are shown in Table 12.

5

Table 12: Polyethylene Glycol Alkyl Ethers

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|----------------------------------|---|-----|
| PEG-2 oleyl ether,oleth-2 | Brij 92/93 (Atlas/ICI) | 4.9 |
| 10 PEG-3 oleyl ether,oleth-3 | Volpo 3 (Crod) | <10 |
| PEG-5 oleyl ether,oleth-5 | Volpo 5 (Crod) | <10 |
| PEG-10 oleyl ether,oleth-10 | Volpo 10 (Crod), Brij 96/97 (Atlas/ICI) | 12 |
| PEG-20 oleyl ether,oleth-20 | Volpo 20 (Crod), Brij 98/99 (Atlas/ICI) | 15 |
| 15 PEG-4 lauryl ether, laureth-4 | Brij 30 (Atlas/ICI) | 9.7 |
| PEG-9 lauryl ether | | >10 |
| PEG-23 lauryl ether, laureth-23 | Brij 35 (Atlas/ICI) | 17 |
| PEG-2 cetyl ether | Brij 52 (ICI) | 5.3 |
| PEG-10 cetyl ether | Brij 56 (ICI) | 13 |
| 20 PEG-20 cetyl ether | Brij 58 (ICI) | 16 |
| PEG-2 stearyl ether | Brij 72 (ICI) | 4.9 |
| PEG-10 stearyl ether | Brij 76 (ICI) | 12 |
| PEG-20 stearyl ether | Brij 78 (ICI) | 15 |
| PEG-100 stearyl ether | Brij 700 (ICI) | >10 |

25

1.13. Sugar Esters

Esters of sugars are suitable surfactants for use in the present invention. Preferred hydrophilic surfactants in this class include sucrose monopalmitate and sucrose monolaurate. Examples of such surfactants are shown in Table 13.

30

1

Table 13: Sugar Ester Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|---------------------------------|---|-----|
| Sucrose distearate | SUCRO ESTER 7 (Gattefosse), Crodesta F-10 (Codia) | 3 |
| Sucrose distearate/monostearate | SUCRO ESTER 11 (Gattefosse), Crodesta F-110 (Codia) | 12 |
| Sucrose dipalmitate | . | 7.4 |
| Sucrose monostearate | Crodesta F-160 (Codia) | 15 |
| Sucrose monopalmitate | SUCRO ESTER 15 (Gattefosse) | >10 |
| Sucrose monolaurate | Saccharose monolaurate 1695 (Mitsubishi-Kasei) | 15 |

10

1.14. Polyethylene Glycol Alkyl Phenols

Several hydrophilic PEG-alkyl phenol surfactants are available, and are suitable for use in the present invention. Examples of these surfactants are shown in Table 14.

15

Table 14: Polyethylene Glycol Alkyl Phenol Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|-------------------------------|---|-----|
| PEG-10-100 nonyl phenol | Triton X series (Rohm & Haas), Igepal CA series (GAF, USA), Antarox CA series (GAF, UK) | >10 |
| PEG-15-100 octyl phenol ether | Triton N-series (Rohm & Haas), Igepal CO series (GAF, USA), Antarox CO series (GAF, UK) | >10 |

1.15. Polyoxyethylene-Polyoxypropylene Block Copolymers

The POE-POP block copolymers are a unique class of polymeric surfactants. The unique structure of the surfactants, with hydrophilic POE and hydrophobic POP moieties in well-defined ratios and positions, provides a wide variety of surfactants suitable for use in the present invention. These surfactants are available under various trade names, including Syperonic PE series (ICI), Pluronic® series (BASF), Emkalyx, Lutrol (BASF), Supronic, Monolan, Pluracare, and Plurodac. The generic term for these polymers is "poloxamer" (CAS 9003-11-6). These polymers have the formula:



where "a" and "b" denote the number of polyoxyethylene and polyoxypropylene units, respectively.

1 Preferred hydrophilic surfactants of this class include Poloxamers 108, 188, 217,
 238, 288, 338, and 407. Preferred hydrophobic surfactants in this class include
 2 Poloxamers 124, 182, 183, 212, 331, and 335.

5 Examples of suitable surfactants of this class are shown in Table 15. Since the
 compounds are widely available, commercial sources are not listed in the Table. The
 compounds are listed by generic name, with the corresponding "a" and "b" values.

Table 15: POE-POP Block Copolymers

| COMPOUND | a, b values in HO(C ₂ H ₄ O) _a (C ₃ H ₆ O) _b (C ₂ H ₄ O) _a H | HLB |
|------------------|---|-----|
| 10 Poloxamer 105 | a = 11 b = 16 | 8 |
| Poloxamer 108 | a = 46 b = 16 | >10 |
| Poloxamer 122 | a = 5 b = 21 | 3 |
| Poloxamer 123 | a = 7 b = 21 | 7 |
| Poloxamer 124 | a = 11 b = 21 | >7 |
| 15 Poloxamer 181 | a = 3 b = 30 | |
| Poloxamer 182 | a = 8 b = 30 | 2 |
| Poloxamer 183 | a = 10 b = 30 | |
| Poloxamer 184 | a = 13 b = 30 | |
| Poloxamer 185 | a = 19 b = 30 | |
| 20 Poloxamer 188 | a = 75 b = 30 | 29 |
| Poloxamer 212 | a = 8 b = 35 | |
| Poloxamer 215 | a = 24 b = 35 | |
| Poloxamer 217 | a = 52 b = 35 | |
| Poloxamer 231 | a = 16 b = 39 | |
| Poloxamer 234 | a = 22 b = 39 | |
| 25 Poloxamer 235 | a = 27 b = 39 | |
| Poloxamer 237 | a = 62 b = 39 | 24 |
| Poloxamer 238 | a = 97 b = 39 | |
| Poloxamer 282 | a = 10 b = 47 | |
| Poloxamer 284 | a = 21 b = 47 | |
| 30 Poloxamer 288 | a = 122 b = 47 | >10 |
| Poloxamer 331 | a = 7 b = 54 | 0.5 |
| Poloxamer 333 | a = 20 b = 54 | |
| Poloxamer 334 | a = 31 b = 54 | |

| | | |
|---|---------------|----------------|
| 1 | Poloxamer 335 | a = 38 b = 54 |
| | Poloxamer 338 | a = 128 b = 54 |
| | Poloxamer 401 | a = 6 b = 67 |
| | Poloxamer 402 | a = 13 b = 67 |
| 5 | Poloxamer 403 | a = 21 b = 67 |
| | Poloxamer 407 | a = 98 b = 67 |

1.16. Sorbitan Fatty Acid Esters

Sorbitan esters of fatty acids are suitable surfactants for use in the present invention. Among these esters, preferred hydrophobic surfactants include sorbitan monolaurate (Arlacel 20), sorbitan monopalmitate (Span-40), sorbitan monooleate (Span-80), sorbitan monostearate, and sorbitan tristearate. Examples of these surfactants are shown in Table 16.

| Table 16: Sorbitan Fatty Acid Ester Surfactants | | |
|---|--------------------------|---|
| | COMPOUND | COMMERCIAL PRODUCT (Supplier) |
| 15 | Sorbitan monolaurate | Span-20 (Atlas/ICI), Crill 1 (Croda), Arlacel 20 (ICI) 8.6 |
| | Sorbitan monopalmitate | Span-40 (Atlas/ICI), Crill 2 (Croda), Nikkol SP-10 (Nikko) 6.7 |
| | Sorbitan monooleate | Span-80 (Atlas/ICI), Crill 4 (Croda), Crill 50 (Croda) 4.3 |
| 20 | Sorbitan monostearate | Span-60 (Atlas/ICI), Crill 3 (Croda), Nikkol SS-10 (Nikko) 4.7 |
| | Sorbitan trioleate | Span-85 (Atlas/ICI), Crill 45 (Croda), Nikkol SO-30 (Nikko) 4.3 |
| | Sorbitan sesquioleate | Arlacel-C (ICI), Crill 43 (Croda), Nikkol SO-15 (Nikko) 3.7 |
| | Sorbitan tristearate | Span-65 (Atlas/ICI) Crill 35 (Croda), Nikkol SS-30 (Nikko) 2.1 |
| | Sorbitan monoisostearate | Crill 6 (Croda), Nikkol SI-10 (Nikko) 4.7 |
| 25 | Sorbitan sesquistearate | Nikkol SS-15 (Nikko) 4.2 |

1.17. Lower Alcohol Fatty Acid Esters

Esters of lower alcohols (C_2 to C_4) and fatty acids (C_8 to C_{18}) are suitable surfactants for use in the present invention. Among these esters, preferred hydrophobic surfactants include ethyl oleate (Crodamol EO), isopropyl myristate (Crodamol IPM), and isopropyl palmitate (Crodamol IPP). Examples of these surfactants are shown in Table 17.

1

Table 17: Lower Alcohol Fatty Acid Ester Surfactants

| COMPOUND | COMMERCIAL PRODUCT (Supplier) | HLB |
|-----------------------|---|-----|
| Ethyl oleate | Crodamol EO (Croda), Nikkol EOO (Nikko) | <10 |
| Isopropyl myristate | Crodamol IPM (Croda) | <10 |
| 5 Isopropyl palmitate | Crodamol IPP (Croda) | <10 |
| Ethyl linoleate | Nikkol VF-E (Nikko) | <10 |
| Isopropyl linoleate | Nikkol VF-IP (Nikko) | <10 |

10

1.18. Ionic Surfactants

Ionic surfactants, including cationic, anionic and zwitterionic surfactants, are suitable hydrophilic surfactants for use in the present invention. Preferred anionic surfactants include fatty acid salts and bile salts. Preferred cationic surfactants include carnitines. Specifically, preferred ionic surfactants include sodium oleate, sodium lauryl sulfate, sodium lauryl sarcosinate, sodium dioctyl sulfosuccinate, sodium cholate, sodium taurocholate; lauroyl carnitine; palmitoyl carnitine; and myristoyl carnitine. Examples of such surfactants are shown in Table 18. For simplicity, typical counterions are shown in the entries in the Table. It will be appreciated by one skilled in the art, however, that any bioacceptable counterion may be used. For example, although the fatty acids are shown as sodium salts, other cation counterions can also be used, such as alkali metal cations or ammonium. Unlike typical non-ionic surfactants, these ionic surfactants are generally available as pure compounds, rather than commercial (proprietary) mixtures. Because these compounds are readily available from a variety of commercial suppliers, such as Aldrich, Sigma, and the like, commercial sources are not generally listed in the Table.

20

Table 18: Ionic Surfactants

| COMPOUND | HLB |
|-------------------------|-----|
| FATTY ACID SALTS | >10 |
| Sodium caproate | |
| Sodium caprylate | |
| Sodium caprate | |
| 30 Sodium laurate | |
| Sodium myristate | |
| Sodium myristolate | |
| Sodium palmitate | |
| Sodium palmitoleate | |

| | | |
|----|--|-----|
| 1 | Sodium oleate | 18 |
| | Sodium ricinoleate | |
| | Sodium linoleate | |
| | Sodium linolenate | |
| | Sodium stearate | |
| 5 | Sodium lauryl sulfate (dodecyl) | 40 |
| | Sodium tetradecyl sulfate | |
| | Sodium lauryl sarcosinate | |
| | Sodium diethyl sulfosuccinate [sodium docusate (Cytec)] | |
| | BILE SALTS | >10 |
| | Sodium cholate | |
| 10 | Sodium taurocholate | |
| | Sodium glycocholate | |
| | Sodium deoxycholate | |
| | Sodium taurodeoxycholate | |
| | Sodium glycodeoxycholate | |
| | Sodium ursodeoxycholate | |
| | Sodium chenodeoxycholate | |
| 15 | Sodium taurochenodeoxycholate | |
| | Sodium glyco cheno deoxycholate | |
| | Sodium cholylsarcosinate | |
| | Sodium N-methyl taurocholate | |
| | Sodium lithocholate | |
| | PHOSPHOLIPIDS | |
| | Egg/Soy lecithin [Epikuron™ (Lucas Meyer), Ovothin™ (Lucas Meyer)] | |
| 20 | Lyo egg/soy lecithin | |
| | Hydroxylated lecithin | |
| | Lysophosphatidylcholine | |
| | Cardiolipin | |
| | Sphingomyelin | |
| | Phosphatidylcholine | |
| | Phosphatidyl ethanolamine | |
| 25 | Phosphatidic acid | |
| | Phosphatidyl glycerol | |
| | Phosphatidyl serine | |
| | PHOSPHORIC ACID ESTERS | |
| | Diethanolammonium polyoxyethylene-10 oleyl ether phosphate | |
| | Esterification products of fatty alcohols or fatty alcohol ethoxylates with phosphoric acid or anhydride | |
| 30 | CARBOXYLATES | |
| | Ether carboxylates (by oxidation of terminal OH group of fatty alcohol ethoxylates) | |
| | Succinylated monoglycerides [LAMEGIN ZE (Henkel)] | |

- 1 Sodium stearyl fumarate

Stearoyl propylene glycol hydrogen succinate

Mono/diacetylated tartaric acid esters of mono- and diglycerides

Citric acid esters of mono-, diglycerides

Glyceryl-lacto esters of fatty acids (CFR ref. 172.852)

5 Acyl lactylates:

 - lauryl esters of fatty acids
 - calcium/sodium stearoyl-2-lactylate
 - calcium/sodium stearoyl lactylate

Alginic salts

Propylene glycol alginate

SULFATES AND SULFONATES

10 Ethoxylated alkyl sulfates

Alkyl benzene sulfones

α -olefin sulfonates

Acyl isethionates

Acyl taurates

Alkyl glyceryl ether sulfonates

Octyl sulfosuccinate disodium

15 Disodium undecylenamideo-MEA-sulfosuccinate

CATIONIC Surfactants

Lauroyl carnitine

Palmitoyl carnitine

Myristoyl carnitine

Hexadecyl triammonium bromide

Decyl trimethyl ammonium bromide

20 Cetyl trimethyl ammonium bromide

Dodecyl ammonium chloride

Alkyl benzyl(dimethyl)ammonium salts

Diisobutyl phenoxyethoxydimethyl benzylammonium salts

Alkylpyridinium salts

25 Betaines (trialkylglycine):

 - Lauryl betaine (N-lauryl,N,N-dimethylglycine)

Ethoxylated amines:

 - Polyoxvethylene-15 coconut amine

1.19 Ionizable Surfactants

Ionizable surfactants, when present in their un-ionized (neutral, non-salt) form, are hydrophobic surfactants suitable for use in the compositions and methods of the present invention, and in their ionized form, are hydrophilic surfactants suitable for use in the present invention. Particular examples of such surfactants include free fatty acids, particularly C₆-C₂₂ fatty acids, and bile acids. More specifically, suitable unionized

1 ionizable surfactants include the free fatty acid and bile acid forms of any of the fatty acid
salts and bile salts shown in Table 18. Preferred ionizable surfactants include fatty acids
and their corresponding salts, such as caprylic acid/sodium caprylate, oleic acid/sodium
oleate, capric acid/sodium caprate; ricinoleic acid/sodium ricinoleate, linoleic acid/sodium
5 linoleate, and lauric acid/sodium laurate; trihydroxy bile acids and their salts, such as
cholic acid (natural), glycocholic acid and taurocholic acid; dihydroxy bile acids and their
salts, such as deoxycholic acid (natural), glycdeoxycholic acid, taurodeoxycholic acid,
chenodeoxycholic acid (natural), glycochenodeoxycholic acid, taurochenodeoxycholic
acid, ursodeoxycholic acid, taouroursodeoxycholic acid, and glycoursodeoxycholic acid;
10 monohydroxy bile acids and their salts, such as lithocholic acid (natural); sulfated bile salt
derivatives; sарchocholate; fusidic acid and its derivatives; phospholipids, such as
phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, PD inisitol,
lysolecithin, and palmitoyl lysophosphatidyl choline; carnitines, such as palmitoyl
15 carnitine, lauroyl carnitine and myristoyl carnitine; cyclodextrins, including alpha, beta
and gamma cyclodextrins; and modified cyclodextrins, such as hydroxy propyl and
sulfobutyl ether.

1.20 Preferred Surfactants and Surfactant Combinations

Among the above-listed surfactants, several combinations are preferred. In all of
the preferred combinations, the absorption enhancing composition includes at least one
20 hydrophilic surfactant. Preferred non-ionic hydrophilic surfactants include
alkylglucosides; alkylmaltosides; alkylthioglucosides; lauryl macrogolglycerides;
polyoxyethylene alkyl ethers; polyoxyethylene alkylphenols; polyethylene glycol fatty
acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty
acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglycerol fatty acid
25 esters; polyoxyethylene glycerides; polyoxyethylene sterols, derivatives, and analogues
thereof; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils;
reaction mixtures of polyols with fatty acids, glycerides, vegetable oils, hydrogenated
vegetable oils, and sterols; sugar esters, sugar ethers; sucroglycerides; and mixtures
thereof.

30 More preferably, the non-ionic hydrophilic surfactant is selected from the group
consisting of polyoxyethylene alkylethers; polyethylene glycol fatty acids esters;
polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters;

- 1 polyoxyethylene-polyoxypropylene block copolymers; polyglyceryl fatty acid esters; polyoxyethylene glycerides; polyoxyethylene vegetable oils; and polyoxyethylene hydrogenated vegetable oils. The glyceride can be a monoglyceride, diglyceride, triglyceride, or a mixture.
- 5 Also preferred are non-ionic hydrophilic surfactants that are reaction mixtures of polyols and fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils or sterols. These reaction mixtures are largely composed of the transesterification products of the reaction, along with often complex mixtures of other reaction products. The polyol is preferably glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, 10 pentaerythritol, or a saccharide.

Several particularly preferred absorption enhancing compositions are those which include as a non-ionic hydrophilic surfactant PEG-10 laurate, PEG-12 laurate, PEG-20 laurate, PEG-32 laurate, PEG-32 dilaurate, PEG-12 oleate, PEG-15 oleate, PEG-20 oleate, PEG-20 dioleate, PEG-32 oleate, PEG-200 oleate, PEG-400 oleate, PEG-15 stearate, 15 PEG-32 distearate, PEG-40 stearate, PEG-100 stearate, PEG-20 dilaurate, PEG-25 glyceryl trioleate, PEG-32 dioleate, PEG-20 glyceryl laurate, PEG-30 glyceryl laurate, PEG-20 glyceryl stearate, PEG-20 glyceryl oleate, PEG-30 glyceryl oleate, PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-40 palm kernel oil, PEG-50 hydrogenated castor oil, PEG-40 castor oil, PEG-35 castor oil, PEG-60 castor oil, PEG-40 hydrogenated 20 castor oil, PEG-60 hydrogenated castor oil, PEG-60 corn oil, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polyglyceryl-10 laurate, PEG-30 cholesterol, PEG-25 phytosterol, PEG-30 soya sterol, PEG-20 trioleate, PEG-40 sorbitan oleate, PEG-80 sorbitan laurate, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, POE-20 oleyl ether, POE-20 stearyl ether, tocopheryl 25 PEG-100 succinate, PEG-24 cholesterol, polyglyceryl-10 oleate, Tween 40, Tween 60, sucrose monostearate, sucrose monolaurate, sucrose monopalmitate, PEG 10-100 nonyl phenol series, PEG 15-100 octyl phenol series, or a poloxamer.

Among these preferred surfactants, more preferred are PEG-20 laurate, PEG-20 oleate, PEG-35 castor oil, PEG-40 palm kernel oil, PEG-40 hydrogenated castor oil, PEG-30 60 corn oil, PEG-25 glyceryl trioleate, polyglyceryl-10 laurate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, PEG-30 cholesterol, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, PEG-24

1 cholesterol, sucrose monostearate, sucrose monolaurate and poloxamers. Most preferred
are PEG-35 castor oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl
trioleate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides,
polysorbate 20, polysorbate 80, tocopheryl PEG-1000 succinate, PEG-24 cholesterol, and
5 hydrophilic poloxamers.

The hydrophilic surfactant can also be, or include as a component, an ionic surfactant, *i.e.*, the ionized form of an ionizable surfactant. Preferred ionic surfactants include the ionized form of alkyl ammonium salts; bile acids and salts, analogues, and derivatives thereof; fusidic acid and derivatives thereof; fatty acid derivatives of amino 10 acids, oligopeptides, and polypeptides; glyceride derivatives of amino acids, oligopeptides, and polypeptides; acyl lactylates; mono-,diacetylated tartaric acid esters of mono-,diglycerides; succinylated monoglycerides; citric acid esters of mono-,diglycerides; alginate salts; propylene glycol alginate; lecithins and hydrogenated lecithins; lysolecithin and hydrogenated lysolecithins; lysophospholipids and derivatives thereof; phospholipids 15 and derivatives thereof; salts of alkylsulfates; salts of fatty acids; sodium docusate; carnitines; and mixtures thereof.

More preferable ionized ionizable surfactants include the ionized form of bile acids and salts, analogues, and derivatives thereof; lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; salts of alkylsulfates; salts of fatty acids; 20 sodium docusate; acyl lactylates; mono-,diacetylated tartaric acid esters of mono-,diglycerides; succinylated monoglycerides; citric acid esters of mono-,diglycerides; carnitines; and mixtures thereof.

More specifically, preferred ionized ionizable surfactants are the ionized forms of 25 lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid, lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine, lactyl esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated 30 monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate, taurodeoxycholate, chenodeoxycholate, glycodeoxycholate, glycochenodeoxycholate, taurochenodeoxycholate, ursodeoxycholate, taouroursodeoxycholate,

- 1 glycoursoodeoxycholate, cholylsarcosine, N-methyl taurocholate, caproate, caprylate, caprate, laurate, myristate, palmitate, oleate, ricinoleate, linoleate, linolenate, stearate, lauryl sulfate, teraceetyl sulfate, docusate, lauroyl carnitines, palmitoyl carnitines, myristoyl carnitines, and salts and mixtures thereof.
- 5 Particularly preferred ionized ionizable surfactants are the ionized forms of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of
- 10 mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate, taurodeoxycholate, glycodeoxycholate, cholylsarcosine, caproate, caprylate, caprate, laurate, oleate, lauryl sulfate, docusate, and salts and mixtures thereof, with the most preferred ionic surfactants being lecithin, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides,
- 15 citric acid esters of mono/diglycerides, taurocholate, caprylate, caprate, oleate, lauryl sulfate, docusate, and salts and mixtures thereof.

The absorption enhancing compositions include at least two surfactants, at least one of which is hydrophilic. In one embodiment, the present invention includes at two surfactants that are hydrophilic, and preferred hydrophilic surfactants are listed above. In
20 another embodiment, the composition includes at least one hydrophilic surfactant and at least one hydrophobic surfactant.

In this embodiment, the hydrophobic surfactant can be an unionized ionizable surfactant. Preferably, the unionized ionizable surfactant is the unionized form of a surfactant selected from the group consisting of bile acids and analogues and derivatives
25 thereof; lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; carnitine fatty acid esters; alkylsulfates; fatty acids; acyl lactylates; mono-,diacetylated tartaric acid esters of mono-,diglycerides; succinylated monoglycerides; citric acid esters of mono-,diglycerides; and mixtures thereof.

More preferably, the un-ionized ionizable surfactant is the un-ionized form of a
30 surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol,

- 1 lysophosphatidic acid, lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-
phosphatidylethanolamine, lactylc esters of fatty acids, stearoyl-2-lactylate, stearoyl
lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of
mono/diglycerides, citric acid esters of mono/diglycerides, cholic acid, taurocholic acid,
5 glycocholic acid, deoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid,
glycodeoxycholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid,
ursodeoxycholic acid, lithocholic acid, tauroursodeoxycholic acid, glycoursoodeoxycholic
acid, cholylsarcosine, N-methyl taurocholic acid, caproic acid, caprylic acid, capric acid,
lauric acid, myristic acid, palmitic acid, oleic acid, ricinoleic acid, linoleic acid, linolenic
10 acid, stearic acid, lauryl sulfate, tetraacetyl sulfate, lauroyl carnitine, palmitoyl carnitine,
myristoyl carnitine, and mixtures thereof.

Still more preferably, the un-ionized ionizable surfactant is the un-ionized form of
a surfactant selected from the group consisting of lecithin, lysolecithin,
15 phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol,
lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactylc esters of fatty acids,
stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated
tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholic
acid, taurocholic acid, glycocholic acid, deoxycholic acid, chenodeoxycholic acid,
lithocholic acid, ursodeoxycholic acid, taurodeoxycholic acid, glycodeoxycholic acid,
20 cholylsarcosine, caproic acid, caprylic acid, capric acid, lauric acid, oleic acid, lauryl
sulfate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and mixtures thereof.

Most preferably, the un-ionized ionizable surfactant is the un-ionized form of a
surfactant selected from the group consisting of lecithin, lactylc esters of fatty acids,
stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated
25 tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides,
chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid, taurocholic acid, caprylic
acid, capric acid, oleic acid, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine,
myristoyl carnitine, and mixtures thereof.

The hydrophobic surfactants can also be alcohols; polyoxyethylene alkylethers;
30 fatty acids; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower alcohol
fatty acids esters; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty
acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; lactic acid

- 1 derivatives of mono/diglycerides; propylene glycol diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; transesterified vegetable oils; sterols; sterol derivatives; sugar esters; sugar ethers; sucroglycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; and the un-ionized (neutral) forms of ionizable surfactants.

5 As with the hydrophilic surfactants, hydrophobic surfactants can be reaction mixtures of polyols and fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

- 10 Preferably, the hydrophobic surfactant is selected from the group consisting of fatty acids; lower alcohol fatty acid esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lactic acid derivatives of mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; and reaction mixtures of polyols and fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

15 More preferred are lower alcohol fatty acids esters; polypropylene glycol fatty acid esters; propylene glycol fatty acid esters; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lactic acid derivatives of mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene vegetable oils; and mixtures thereof, with glycerol fatty acid esters and acetylated glycerol fatty acid esters being most preferred. Among the glycerol fatty acid esters, the esters are preferably mono- or diglycerides, or mixtures of mono- and diglycerides, where the fatty acid moiety is a C₆ to C₂₂ fatty acid.

20 Also preferred are hydrophobic surfactants which are the reaction mixture of polyols and fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols. Preferred polyols are polyethylene glycol, sorbitol, propylene glycol, and pentaerythritol.

25 Specifically preferred hydrophobic surfactants include myristic acid; oleic acid; lauric acid; stearic acid; palmitic acid; PEG 1-4 stearate; PEG 2-4 oleate; PEG-4 dilaurate; PEG-4 dioleate; PEG-4 distearate; PEG-6 dioleate; PEG-6 distearate; PEG-8 dioleate; PEG 3-16 castor oil; PEG 5-10 hydrogenated castor oil; PEG 6-20 corn oil; PEG 6-20 almond oil; PEG-6 olive oil; PEG-6 peanut oil; PEG-6 palm kernel oil; PEG-6

- 1 hydrogenated palm kernel oil; PEG-4 capric/caprylic triglyceride, mono, di, tri, tetra esters
of vegetable oil and sorbitol; pentaerythrityl di, tetra stearate, isostearate, oleate, caprylate,
or caprate; polyglyceryl 2-4 oleate, stearate, or isostearate; polyglyceryl 4-10 pentaoleate;
polyglyceryl-3 dioleate; polyglyceryl-6 dioleate; polyglyceryl-10 trioleate; polyglyceryl-3
5 distearate; propylene glycol mono- or diesters of a C₆ to C₂₀ fatty acid; monoglycerides of
C₆ to C₂₀ fatty acids; acetylated monoglycerides of C₆ to C₂₀ fatty acids; diglycerides of C₆
to C₂₀ fatty acids; lactic acid derivatives of monoglycerides; lactic acid derivatives of
diglycerides; cholesterol; phytosterol; PEG 5-20 soya sterol; PEG-6 sorbitan tetra,
hexastearate; PEG-6 sorbitan tetraoleate; sorbitan monolaurate; sorbitan monopalmitate;
10 sorbitan mono, trioleate; sorbitan mono, tristearate; sorbitan monoisostearate; sorbitan
sesquioleate; sorbitan sesquistearate; PEG 2-5 oleyl ether; POE 2-4 lauryl ether; PEG-2
cetyl ether; PEG-2 stearyl ether; sucrose distearate; sucrose dipalmitate; ethyl oleate;
isopropyl myristate; isopropyl palmitate; ethyl linoleate; isopropyl linoleate; and
poloxamers.
- 15 Among the specifically preferred hydrophobic surfactants, most preferred are oleic
acid; lauric acid; glyceryl monocaprate; glyceryl monocaprylate; glyceryl monolaurate;
glyceryl monooleate; glyceryl dicaprate; glyceryl dicaprylate; glyceryl dilaurate; glyceryl
dioleate; acetylated monoglycerides; propylene glycol oleate; propylene glycol laurate;
polyglyceryl-3 oleate; polyglyceryl-6 dioleate; PEG-6 corn oil; PEG-20 corn oil; PEG-20
20 almond oil; sorbitan monooleate; sorbitan monolaurate; POE-4 lauryl ether; POE-3 oleyl
ether; ethyl oleate; and poloxamers.

2. Therapeutic Agents

The hydrophilic therapeutic agents suitable for use in the pharmaceutical systems
and methods of the present invention are not particularly limited, as the absorption
25 enhancing compositions are surprisingly capable of delivering a wide variety of
hydrophilic therapeutic agents. Suitable hydrophilic therapeutic agents include
hydrophilic drugs (*i.e.*, conventional non-peptidic drugs), hydrophilic macromolecules
such as cytokines, peptidomimetics, peptides, proteins, toxoids, sera, antibodies, vaccines,
nucleosides, nucleotides and genetic material, and other hydrophilic compounds, such as
30 nucleic acids. The aqueous solubility of the hydrophilic therapeutic agent should be
greater than about 1 mg/mL.

1 The hydrophilic therapeutic agent can be solubilized or suspended in a
preconcentrate (before dilution with an aqueous diluent), added to the preconcentrate prior
to dilution, added to the diluted preconcentrate, or added to an aqueous diluent prior to
mixing with the preconcentrate. The hydrophilic therapeutic agent can also be co-
5 administered as part of an independent dosage form, for therapeutic effect. Optionally, the
hydrophilic therapeutic agent can be present in a first, solubilized amount, and a second,
non-solubilized (suspended) amount. Such hydrophilic therapeutic agents can be any
agents having therapeutic or other value when administered to an animal, particularly to a
mammal, such as drugs, nutrients, cosmetics (cosmeceuticals), and diagnostic agents. It
10 should be understood that while the invention is described with particular reference to its
value for oral dosage forms, the invention is not so limited. Thus, hydrophilic drugs,
nutrients, cosmetics and diagnostic agents which derive their therapeutic or other value
from, for example, transmembrane (transport across a membrane barrier of therapeutic
significance), nasal, buccal, rectal, vaginal or pulmonary administration, are still
15 considered to be suitable for use in the present invention.

Specific non-limiting examples of therapeutic agents that can be used in the pharmaceutical compositions of the present invention include analgesics and anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, anti-asthma agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents and immunosuppressants, anti-protozoal agents, anti-thyroid agents, anti-tussives, anxiolytic, sedatives, hypnotics and neuroleptics, β -Blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonian agents, gastro-intestinal agents, histamine H₂ receptor antagonists, keratolytics, lipid regulating agents, muscle relaxants, anti-anginal agents, nutritional agents, analgesics, sex hormones, stimulants, cytokines, peptidomimetics, peptides, proteins, toxoids, sera, antibodies, vaccines, nucleosides, nucleotides and genetic material, and nucleic acids. Amphiphilic therapeutic agents are also included, provided they have a water solubility of greater than about 1 mg/mL.

30 In one embodiment, the hydrophilic therapeutic agent is a nutritional agent.

 In another embodiment, the hydrophilic therapeutic agent is a cosmeceutical agent.

 In another embodiment, the hydrophilic therapeutic agent is a diagnostic agent.

- 1 Although the invention is not limited thereby, examples of hydrophilic therapeutic
agents suitable for use in the compositions and methods of the present invention include
the following preferred compounds, as well as their pharmaceutically acceptable salts,
isomers, esters, ethers and other derivatives:
- 5 acarbose; acyclovir; acetyl cysteine; acetylcholine chloride; alatrofloxacin;
alendronate; alglucerase; amantadine hydrochloride; ambenonium; amifostine; amiloride
hydrochloride; aminocaproic acid; amphotericin B; antihemophilic factor (human);
antihemophilic factor (porcine); antihemophilic factor (recombinant); aprotinin;
asparaginase; atenolol; atracurium besylate; atropine; azithromycin; aztreonam; BCG
10 vaccine; bacitracin; becalermin; belladona; bepridil hydrochloride; bleomycin sulfate;
calcitonin human; calcitonin salmon; carboplatin; capecitabine; capreomycin sulfate;
cefamandole nafate; cefazolin sodium; cefepime hydrochloride; cefixime; cefonicid
sodium; cefoperazone; cefotetan disodium; cefotoxime; cefoxitin sodium; ceftriaxime;
ceftriaxone; cefuroxime axetil; cephalexin; cephalpirin sodium; cholera vaccine; chrinic
15 gonadotropin; cidofovir; cisplatin; cladribine; clidinium bromide; clindamycin and
clindamycin derivatives; ciprofloxacin; clondronate; colistimethate sodium; colistin
sulfate; corticotropin; cosyntropin; cromalyin sodium; cytarabine; daltaperin sodium;
danaproid; deforoxamine; denileukin diftitox; desmopressin; diatrizoate meglumine and
diatrizoate sodium; dicyclomine; didanosine; dirithromycin; dopamine hydrochloride;
20 domase alpha; doxacurium chloride; doxorubicin; editronate disodium; elanaprilat;
enkephalin; enoxacin; enoxaprin sodium; ephedrine; epinephrine; epoetin alpha;
erythromycin; esmol hydrochloride; factor IX; famciclovir; fludarabine; fluoxetine;
foscarnet sodium; ganciclovir; granulocyte colony stimulating factor; granulocyte-
macrophage stimulating factor; growth hormones- recombinant human; growth
25 hormone- bovine; gentamycin; glucagon; glycopyrrolate; gonadotropin releasing hormone
and synthetic analogs thereof; GnRH; gonadorelin; grepafloxacin; hemophilus B conjugate
vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated;
heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3;
insulin-human; insulin lispro; insulin procine; insulin NPH; insulin aspart; insulin
30 glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide;
isofosfamide; japanese encephalitis virus vaccine; lamivudine; leucovorin calcium;
leuprolide acetate; levofloxacin; lincomycin and lincomycin derivatives; lobucavir;

- 1 lomefloxacin; loracarbef; mannitol; measles virus vaccine; meningococcal vaccine;
menotropins; mephenzolate bromide; mesalmine; methanamine; methotrexate;
methscopolamine; metformin hydrochloride; metroprolol; mezocillin sodium; mivacurium
chloride; mumps viral vaccine; nedocromil sodium; neostigmine bromide; neostigmine
5 methyl sulfate; neutontin; norfloxacin; octreotide acetate; ofloxacin; olpadronate;
oxytocin; pamidronate disodium; pancuronium bromide; paroxetine; pefloxacin;
pentamidine isethionate; pentostatin; pentoxifylline; periclovir; pentagastrin;
phenotolamine mesylate; phenylalanine; physostigmine salicylate; plague vaccine;
piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine
10 polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymixin B
sulfate; pralidoxine chloride; pramlintide; pregabalin; propofenone; propenthaline
bromide; pyridostigmine bromide; rabies vaccine; residronate; ribavarin; rimantadine
hydrochloride; rotavirus vaccine; salmetrol xinafoate; sincalide; small pox vaccine;
solatol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin;
15 suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiopeta; ticarcillin;
tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; trandolapril;
trimetrexate gluconate; trospectinomycin; trovafloxacin; tubocurarine chloride; tumor
necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valaciclovir; valsartan;
varicella virus vaccine live; vasopressin and vasopressin derivatives; vecuronium bromide;
20 vinblastin; vincristine; vinorelbine; vitamin B12 ; warfarin sodium; yellow fever vaccine;
zalcitabine; zanamavir; zolandronate; and zidovudine.

Among the listed hydrophilic therapeutic agents, more preferred therapeutic agents
are:

- 25 acarbose; acyclovir; atracurium besylate; alendronate; alglycerase; amantadine
hydrochloride; amphotericin B; antihemophilic factor (human); antihemophilic factor
(porcine); antihemophilic factor (recombinant; azithromycin; calcitonin human; calcitonin
salmon; capecitabine; cefazolin sodium; cefonicid sodium; cefoperazone; cefoxitin
sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalexin; chrionic gonadotropin;
cidofovir; cladribine ; clindamycin and clindamycin derivatives; corticotropin;
30 cosyntropin; cromalyn sodium; cytarabine; daltaperin sodium; danaproid; desmopressin;
didanosine; dirithromycin; editronate disodium; enoxaprin sodium; epoetin alpha; factor
IX; famciclovir; fludarabine; foscarnet sodium; ganciclovir; granulocyte colony

1 stimulating factor; granulocyte-macrophage stimulating factor; growth hormones-
recombinant human; growth hormone- Bovine; gentamycin; glucagon; gonadotropin
releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; hemophilus B
conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine
5 inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2;
interleukin-3; insulin-human; insulin lispro; insulin procine; insulin NPH; insulin aspart;
insulin glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide;
isofosfamide; lamivudine; leucovorin calcium; leuprolide acetate; lincomycin and
lincomycin derivatives; metformin hydrochloride; nedocromil sodium; neostigmine
10 bromide; neostigmine methyl sulfate; neutontin; octreotide acetate; olpadronate;
pamidronate disodium; pancuronium bromide; pentamidine isethionate; pentagastrin;
physostigmine salicylate; poliovirus vaccine live (OPV); pyridostigmine bromide;
residronate; ribavarin; rimantadine hydrochloride; rotavirus vaccine; salmetrol xinafoate;
somatostatin; spectinomycin; stavudine; streptokinase; ticarcillin; tiludronate; tissue type
15 plasminogen activator; TNFR:Fc; TNK-tPA; trimetrexate gluconate; prospectinomycin;
tumor necrosis factor; typhoid vaccine live; urokinase; vancomycin; valaciclovir;
vasopressin and vasopressin derivatives; vinblastin; vincristine; vinorelbine; warfarin
sodium; zalcitabine; zanamavir; and zidovudine.

The most preferred hydrophilic therapeutic agents are:

20 acarbose; alendronate; amantadine hydrochloride; azithromycin; calcitonin human;
calcitonin salmon; ceftriaxone; cefuroxime axetil; chrinic gonadotropin; cromalyn
sodium; daltaperin sodium; danaproid; desmopressin; didanosine; editronate disodium;
enoxaprin sodium; epoetin alpha; factor IX; famiciclovir; foscarnet sodium; ganciclovir;
granulocyte colony stimulating factor; granulocyte-macrophage stimulating factor; growth
25 hormones- recombinant human; growth hormone- Bovine; glucagon; gonadotropin
releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; heparin sodium;
indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human;
insulin lispro; insulin procine interferon alpha; interferon beta; leuprolide acetate;
metformin hydrochloride; nedocromil sodium; neostigmine bromide; neostigmine methyl
30 sulfate; neutontin; octreotide acetate; olpadronate; pamidronate disodium; residronate;
rimantadine hydrochloride; salmetrol xinafoate; somatostatin; stavudine; ticarcillin;
tiludronate; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; tumor necrosis

1 factor; typhoid vaccine live; vancomycin; valaciclovir; vasopressin and vasopressin derivatives; zalcitabine; zanamavir and zidovudine.

2 Of course, salts, metabolic precursors, derivatives and mixtures of therapeutic agents may also be used where desired.

3. **Solubilizers**

If desired, the pharmaceutical compositions of the present invention can optionally include additional compounds to enhance the solubility of the therapeutic agent or the triglyceride in the composition. Examples of such compounds, referred to as "solubilizers", include:

10 alcohols and polyols, such as ethanol, isopropanol, butanol, benzyl alcohol, ethylene glycol, propylene glycol, butanediols and isomers thereof, glycerol, pentaerythritol, sorbitol, mannitol, transcutol, dimethyl isosorbide, polyethylene glycol, polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other cellulose derivatives, cyclodextrins and cyclodextrin derivatives;

15 ethers of polyethylene glycols having an average molecular weight of about 200 to about 6000, such as tetrahydrofuryl alcohol PEG ether (glycofurol, available commercially from BASF under the trade name Tetraglycol) or methoxy PEG (Union Carbide);

20 amides, such as 2-pyrrolidone, 2-piperidone, ϵ -caprolactam, N-alkylpyrrolidone, N-hydroxalkylpyrrolidone, N-alkylpiperidone, N-alkylcaprolactam, dimethylacetamide, and polyvinylpyrrolidone;

25 esters, such as ethyl propionate, tributylcitrate, acetyl triethylcitrate, acetyl tributyl citrate, triethylcitrate, ethyl oleate, ethyl caprylate, ethyl butyrate, triacetin, propylene glycol monoacetate, propylene glycol diacetate, ϵ -caprolactone and isomers thereof, δ -valerolactone and isomers thereof, β -butyrolactone and isomers thereof;

30 and other solubilizers known in the art, such as dimethyl acetamide, dimethyl isosorbide (Arlasolve DMI (ICI)), N-methyl pyrrolidones (Pharmasolve (ISP)), monooctanoin, diethylene glycol monoethyl ether (available from Gattefosse under the trade name Transcutol), and water.

Mixtures of solubilizers are also within the scope of the invention. Except as indicated, these compounds are readily available from standard commercial sources.

1 Preferred solubilizers include triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cyclodextrins, ethanol, polyethylene glycol 200-100, glycofurool, transcutol, propylene glycol, and 5 dimethyl isosorbide. Particularly preferred solubilizers include sorbitol, glycerol, triacetin, ethyl alcohol, PEG-400, glycofurool and propylene glycol.

The amount of solubilizer that can be included in compositions of the present invention is not particularly limited. Of course, when such compositions are ultimately administered to a patient, the amount of a given solubilizer is limited to a bioacceptable 10 amount, which is readily determined by one of skill in the art. In some circumstances, it may be advantageous to include amounts of solubilizers far in excess of bioacceptable amounts, for example, to maximize the concentration of therapeutic agent, with excess solubilizer removed prior to providing the composition to a patient using conventional techniques, such as distillation or evaporation. Thus, if present, the solubilizer can be in a 15 concentration of 50%, 100%, 200%, or up to about 400% by weight, based on the weight of the carrier. If desired, very small amounts of solubilizers may also be used, such as 25%, 10%, 5%, 1% or even less. Typically, the solubilizer will be present in an amount of about 1% to about 100%, more typically about 5% to about 25% by weight or about 10% to about 25% by weight.

20 **4. Concentrations**

The components of the absorption enhancing compositions of the present invention are present in amounts such that upon dilution with an aqueous diluent, the carrier forms an aqueous dispersion having a small particle size. The hydrophilic and optional 25 hydrophobic surfactants should be present in amounts sufficient to improve the absorption of the hydrophilic therapeutic agent. It is surprisingly found that relatively large amounts of the surfactants can be used while still maintaining a small particle size upon dilution.

Without wishing to be bound by theory, it is believed that the absorption enhancers present in the compositions are able to enhance absorption by one or more of the following factors: effective presentation of an absorption enhancer to the site of enhancement; 30 modulation of facilitated/active transport; transcellular permeability enhancement through favorable membrane perturbations; inhibition of efflux related transporters; inhibition of luminal or cellular enzymatic inactivation; paracellular transport enhancement through

1 loosening of tight junctions; induction of specific transporters to facilitate transport; altered biological binding characteristics; reduced degradation of the hydrophilic therapeutic agent; induction of transient water channels; and/or increased partitioning of
5 the hydrophilic therapeutic agent by association with the absorption enhancer. The functionality is believed to be due to a combination of small particle size, appropriate absorption enhancers in amounts chosen to provide small particle size upon dilution, and non-dependence upon lipolysis by avoiding the use of triglycerides. Preferably, diesters of propylene glycol are also avoided.

10 The presence of at least two surfactants, at least one of which is hydrophilic, is believed to be particularly advantageous to provide better presentation of the absorption enhancing components at the absorption site. For example, the presence of each surfactant is believed to assist the absorption enhancement functionality of the other surfactants by reducing the size of the particles containing the absorption enhancing surfactant to minimize aqueous boundary layer control, and/or by solubilizing water-
15 immiscible absorption enhancing surfactants to increase the thermodynamic activity of the surfactant at the absorption site.

20 A preferred method of assessing the appropriate component concentrations is to quantitatively measure the size of the particles of which the dispersion is composed. These measurements can be performed on commercially available particle size analyzers, such as, for example, a Nicomp particle size analyzer available from Particle Size Systems, Inc., of Santa Barbara, CA. Using this measure, aqueous dispersions according to the present invention have average particle sizes much smaller than the wavelength of visible light, whereas dispersions containing relative amounts of the components outside the appropriate range have more complex particle size distributions, with much greater
25 average particle sizes. It is desirable that the average particle size be less than about 200 nm, preferably less than about 100, more preferably less than about 50 nm, still more preferably less than about 30 nm, and most preferably less than about 20 nm. It is also preferred that the particle size distribution be mono-modal. These particle sizes can be measured at dilution amounts of 10 to 250-fold or more, preferably about 100 to about
30 250-fold, as is typical of the dilution expected in the gastrointestinal tract.

In a preferred embodiment, the components of the absorption enhancing compositions are present in amounts such that the aqueous dispersion formed upon

1 dilution with an aqueous medium has a small particle size and is also substantially
optically clear. The composition in the preconcentrate form, *i.e.*, before dilution with an
aqueous diluent, need not be clear, as it is the clarity upon dilution with an aqueous diluent
that is preferred. The dilution can be *in vitro* or *in vivo*, and optical clarity should be
5 assessed at dilutions of about 10 to 250-fold or more, preferably about 100 to 250-fold, as
is encountered in the gastrointestinal environment. It should be appreciated that when the
desired dosage form includes an amount of the hydrophilic therapeutic agent that is
suspended, but not solubilized, in the composition, the appropriate concentrations of the
other components are determined by the optical clarity of the diluted composition without
10 the suspended therapeutic agent.

In this preferred embodiment, the relative amounts of the components are readily
determined by observing the properties of the resultant dispersion; *i.e.*, when the relative
amounts are within the preferred range, the resultant aqueous dispersion is optically clear.
When the relative amounts are outside the preferred range, the resulting dispersion is
15 visibly "cloudy", resembling a conventional emulsion or multiple-phase system. The
optical clarity of the aqueous dispersion can be measured using standard quantitative
techniques for turbidity assessment. One convenient procedure to measure turbidity is to
measure the amount of light of a given wavelength transmitted by the solution, using, for
example, a UV-visible spectrophotometer. Using this measure, optical clarity corresponds
20 to high transmittance, since cloudier solutions will scatter more of the incident radiation,
resulting in lower transmittance measurements. If this procedure is used, care should be
taken to insure that the composition itself does not absorb light of the chosen wavelength,
as any true absorbance necessarily reduces the amount of transmitted light and falsely
increases the quantitative turbidity value. In the absence of chromophores at the chosen
25 wavelength, suitable dispersions at a dilution of 100X should have an apparent absorbance
of less than about 0.3, preferably less than about 0.2, and more preferably less than about
0.1.

Other methods of characterizing optical clarity known in the art may also be used,
and any or all of the available methods may be used to ensure that the resulting aqueous
30 dispersions possess the preferred optical clarity.

In one embodiment, the hydrophilic therapeutic agent is formulated in the dosage
form of the absorption enhancing composition, and is present in any amount up to the

1 maximum amount that can be solubilized in the composition. In another embodiment, the hydrophilic therapeutic agent is present in the dosage form of the absorption enhancing composition in a first amount which is solubilized, and a second amount that remains unsolubilized but dispersed. This may be desirable when, for example, a larger dose of the
5 hydrophilic therapeutic agent is desired. Of course, in this embodiment, the optical clarity or particle size of the resultant aqueous dispersion is determined before the second non-solubilized amount of the hydrophilic therapeutic agent is added. In another embodiment, the hydrophilic therapeutic agent is present in a dosage form separate from the dosage form of the absorption enhancing composition, and the amount of hydrophilic therapeutic
10 agent is any convenient amount that can be formulated in the separate dosage form, such as a therapeutically effective amount. This separate dosage form of the hydrophilic therapeutic agent can be a dosage form of the present invention, or any conventional dosage form, preferably triglyceride free, such as a commercial dosage form.

15 Other considerations well known to those skilled in the art will further inform the choice of specific proportions of the components. These considerations include the degree of bioacceptability of the compounds, and the desired dosage of hydrophilic therapeutic agent to be provided.

20 Keeping the considerations discussed above in mind, it is important that the composition include sufficient amounts of the absorption enhancing components to provide a therapeutically meaningful increase in the rate and/or extent of bioabsorption. Thus, in general the total amount of absorption enhancing components forming the carrier should be at least about 10% by weight, preferably at least about 20%, based on the total weight of the preconcentrate composition. As shown in the examples herein, the total amount of the absorption enhancing components can be far greater than 20%, and these
25 compositions are also within the scope of the present invention.

It is preferred that when the absorption enhancing composition includes at least two surfactants selected from the group consisting of sodium lauryl sulfate, oleic acid, linoleic acid, monoolein, lecithin, lysolecithin, deoxycholate, taurodeoxycholate, glycochenodeoxycholate, polyoxyethylene X-lauryl ether, where X is from 9 to 20,
30 sodium tauro-24,25-dihydrofusidate, polyoxyethylene ether, polyoxyethylene sorbitan esters, p-t-octylphenoxypolyoxyethylene, N-lauryl- β -D-maltopyranoside, 1-dodecylazacycloheptane-2-azone, and phospholipids, each surfactant is present in an

- 1 amount of greater than 10% by weight, based on the total weight of the pharmaceutical system.

5 Alternatively, appropriate coating can be applied to the dosage form to enable sufficient concentration/amount of the absorption enhancing surfactant/therapeutic agent/inhibitor at the site of absorption.

5. Stability

5.1 Enzyme Inhibitors

When the hydrophilic therapeutic agent is subject to enzymatic degradation, the compositions can include an enzyme inhibiting agent as an absorption enhancing agent. 10 Enzyme inhibiting agents are shown for example, in Bernskop-Schnurch, A., "The use of inhibitory agents to overcome enzymatic barrier to perorally administered therapeutic peptides and proteins", *J. Controlled Release* 52, 1-16 (1998), the disclosure of which is incorporated herein by reference.

15 Generally, inhibitory agents can be divided into the following classes:
Inhibitors that are not based on amino acids, such as P-aminobenzamidine, FK-448, camostat mesylate, sodium glycocholate;

Amino acids and modified amino acids, such as aminoboronic acid derivatives and n-acetylcysteine;

20 Peptides and modified peptides, such as bacitracin, phosphinic acid dipeptide derivatives, pepstatin, antipain, leupeptin, chymostatin, elastatin, bestatin, phosphoramidon, puromycin, cytochalasin potatocarboxy peptidase inhibitor, and amastatin;

25 Polypeptide protease inhibitors, such as aprotinin (bovine pancreatic trypsin inhibitor), Bowman-Birk inhibitor and soybean trypsin inhibitor, chicken egg white trypsin inhibitor, chicken ovoinhibitor, and human pancreatic trypsin inhibitor;

Complexing agents, such as EDTA, EGTA, 1,10- phenanthroline and hydroxychinoline; and

30 Mucoadhesive polymers and polymer-inhibitor conjugates, such as polyacrylate derivatives, chitosan, cellulosics, chitosan-EDTA, chitosan-EDTA-antipain, polyacrylic acid-bacitracin, carboxymethyl cellulose-pepstatin, polyacrylic acid-Bowman-Birk inhibitor.

1 The choice and levels of the enzyme inhibitor are based on toxicity, specificity of
the proteases and the potency of the inhibition. Enteric coated compositions of the present
invention protect hydrophilic therapeutic peptides or proteins in a restricted area of drug
liberation and absorption, and reduce or even exclude extensive dilution effects. The
5 inhibitor can be suspended or solubilized in the composition preconcentrate, or added to
the aqueous diluent or as a beverage.

Without wishing to be bound by theory, it is believed that an inhibitor can function solely or in combination as:

10 a competitive inhibitor, by binding at the substrate binding site of the enzyme,
thereby preventing the access to the substrate; examples of inhibitors believed to operate
by this mechanism are antipain, elastatinal and the Bowman Birk inhibitor;

 a non-competitive inhibitor which can be simultaneously bound to the enzyme site
along with the substrate, as their binding sites are not identical; and/or

15 a complexing agent due to loss in enzymatic activity caused by deprivation of
essential metal ions out of the enzyme structure.

5.2 Water-Free Preconcentrates

In a particular embodiment, the preconcentrate absorption enhancing composition--*i.e.*, the composition before dispersion in an aqueous medium-- is free of water. Water-free compositions are preferred to increase the physical and/or chemical stability of the
20 composition or of individual components thereof, allowing for longer storage. In addition, water-free compositions offer advantages in processing, such as, for example, ease in encapsulation.

6. Other Additives

Other additives conventionally used in pharmaceutical compositions can be
25 included, and these additives are well known in the art. Such additives include
detackifiers, anti-foaming agents, buffering agents, antioxidants, preservatives, chelating
agents, viscomodulators, tonicifiers, flavorants, colorants odorants, opacifiers, suspending
agents, binders, fillers, plasticizers, lubricants, and mixtures thereof. The amounts of such
30 additives can be readily determined by one skilled in the art, according to the particular
properties desired.

An acid or a base may be added to the composition to facilitate processing, or to
prevent degradation of the hydrophilic therapeutic agent. Examples of pharmaceutically

1 acceptable bases include amino acids, amino acid esters, ammonium hydroxide, potassium hydroxide, sodium hydroxide, sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate, magnesium hydroxide, magnesium aluminum silicate, synthetic aluminum silicate, synthetic hydrotalcite, magnesium aluminum hydroxide, diisopropylethylamine, ethanolamine, ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, and the like. Also suitable are bases which are salts of a pharmaceutically acceptable acid, such as acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, amino acids, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, fatty acids, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, uric acid, and the like. Salts of polyprotic acids, such as sodium phosphate, disodium hydrogen phosphate, and sodium dihydrogen phosphate can also be used. When the base is a salt, the cation can be any convenient and pharmaceutically acceptable cation, such as ammonium, alkali metals, alkaline earth metals, and the like. Preferred cations include sodium, potassium, lithium, magnesium, calcium and ammonium.

20 Suitable acids are pharmaceutically acceptable organic or inorganic acids. Examples of suitable inorganic acids include hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, nitric acid, boric acid, phosphoric acid, and the like. Examples of suitable organic acids include acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, amino acids, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, fatty acids, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, uric acid and the like.

25 Although a wide variety of absorption enhancing components, solubilizers and additives can be used in the pharmaceutical systems of the present invention, in one embodiment, it is preferred that the composition be water-free in the preconcentrate form. In another embodiment, it is preferred that the composition be free of propylene glycol diesters. In another embodiment, it is preferred that the composition be free of cholesterol.

1 Of course, combinations of these preferred embodiments are also within the scope of the invention, so that the composition may, for example, be free of several or all of water, propylene glycol diesters and cholesterol.

7. Dosage Forms

5 The pharmaceutical compositions of the present invention can be formulated as a preconcentrate in a liquid, semi-solid, or solid form, or as an aqueous or organic diluted preconcentrate. In the diluted form, the diluent can be water, an aqueous solution, a buffer, an organic solvent, a beverage, a juice, or mixtures thereof. If desired, the diluent can include components soluble therein, such as a hydrophilic therapeutic agent, an 10 enzyme inhibitor, solubilizers, additives, and the like.

The compositions can be processed according to conventional processes known to those skilled in the art, such as lyophilization, encapsulation, compression, melting, extrusion, balling, drying, chilling, molding, spraying, spray congealing, coating, comminution, mixing, homogenization, sonication, cryopelletization, spheronization, and 15 granulation, to produce the desired dosage form.

The dosage form is not particularly limited. Thus, compositions of the present invention can be formulated as pills, capsules, caplets, tablets, granules, pellets, beads or powders. Granules, pellets, beads and powders can, of course, be further processed to form pills, capsules, caplets or tablets.

20 The dosage form can be designed for immediate release, controlled release, extended release, delayed release or targeted delayed release. The definitions of these terms are known to those skilled in the art. Furthermore, the dosage form release profile can be effected by a polymeric matrix composition, a coated matrix composition, a multiparticulate composition, a coated multiparticulate composition, an ion-exchange 25 resin-based composition, an osmosis-based composition, or a biodegradable polymeric composition. Without wishing to be bound by theory, it is believed that the release may be effected through favorable diffusion, dissolution, erosion, ion-exchange, osmosis or combinations thereof.

When formulated as a capsule, the capsule can be a hard or soft gelatin capsule, a 30 starch capsule, or a cellulosic capsule. Such dosage forms can further be coated with, for example, a seal coating, an enteric coating, an extended release coating, or a targeted delayed release coating.

1 The term "extended release coating" as used herein means a coating designed to
effect the delivery of a hydrophilic therapeutic agent, an enzyme inhibitor, or the carrier,
over an extended period of time. Preferably, the extended release coating is a pH-
independent coating formed of, for example, ethyl cellulose, hydroxypropyl cellulose,
5 methylcellulose, hydroxymethyl cellulose, hydroxyethyl cellulose, acrylic esters, or
sodium carboxymethyl cellulose. Various extended release dosage forms can be readily
designed by one skilled in art to achieve delivery of a hydrophilic therapeutic agent, an
absorption enhancing carrier or an enzyme inhibitor to both the small and large intestines,
10 to only the small intestine, or to only the large intestine, depending upon the choice of
coating materials and/or coating thickness.

Dosage forms of the compositions of the present invention can also be formulated
as enteric coated delayed release oral dosage forms, *i.e.*, as an oral dosage form of a
pharmaceutical composition as described herein which utilizes an enteric coating to effect
release of a hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing
15 carrier in the lower gastrointestinal tract. The enteric coated dosage form may be a
compressed or molded or extruded tablet/mold (coated or uncoated) containing granules,
pellets, beads or particles of the hydrophilic therapeutic agent, enzyme inhibitor and/or
absorption enhancing carrier, which are themselves coated or uncoated. The enteric coated
oral dosage form may also be a capsule (coated or uncoated) containing pellets, beads or
20 granules of the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption
enhancing carrier which are themselves coated or uncoated.

The term "enteric coating" as used herein relates to a mixture of pharmaceutically
acceptable excipients which is applied to, combined with, mixed with or otherwise added
to the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier.
25 The coating may be applied to a compressed or molded or extruded tablet, a gelatin
capsule, and/or pellets, beads, granules or particles of the hydrophilic therapeutic agent,
enzyme inhibitor and/or absorption enhancing carrier. The coating may be applied through
an aqueous dispersion or after dissolving in appropriate solvent. Additional additives and
their levels, and selection of a primary coating material or materials will depend on the
30 following properties:

1. resistance to dissolution and disintegration in the stomach;

- 1 2. impermeability to gastric fluids and drug/carrier/enzyme while in the
stomach;
3. ability to dissolve or disintegrate rapidly at the target intestine site;
4. physical and chemical stability during storage;
5. non-toxicity;
6. easy application as a coating (substrate friendly); and
7. economical practicality.

The term "delayed release" as used herein refers to the delivery of the hydrophilic therapeutic agent, an enzyme inhibitor, and/or the absorption enhancing carrier, which is effected by formulating the composition so that the release can be accomplished at some generally predictable location in the lower intestinal tract more distal to that which would have been accomplished if there had been no delayed release alterations. The preferred method for delay of release is coating. Coating prevents exposure of the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier to the epithelial and mucosal tissue of the buccal cavity, pharynx, esophagus, and stomach, and to the enzymes associated with these tissues. This helps to protect the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier and the tissues from any adverse event prior to the delivery at the desired site of absorption. Furthermore, coated compositions of the present invention allow balancing enhancement effectiveness, active protection, and safety liability through coating controlled dilution of the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier upon administration through delayed release or sustained release. Multiple enteric coatings targeted to release hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier at various regions in the lower gastrointestinal tract would enable even more effective and sustained improved delivery throughout the lower gastrointestinal tract.

Any coatings should be applied to a sufficient thickness such that the entire coating does not dissolve in the gastrointestinal fluids at pH below about 5, but does dissolve at pH about 5 and above. It is expected that any anionic polymer exhibiting a pH-dependent solubility profile can be used as an enteric coating in the practice of the present invention to achieve delivery of the hydrophilic therapeutic agent, enzyme inhibitor and/or absorption enhancing carrier to the lower gastrointestinal tract. The coating chosen should

- 1 be compatible with the hydrophilic therapeutic agent and the other selected components. The preferred polymers for use in the present invention are anionic carboxylic polymers. The more preferred polymers and compatible mixtures thereof, and some of their properties, include, but are not limited to:
- 5 Shellac, also called purified lac, a refined product obtained from the resinous secretion of an insect. This coating dissolves in media of pH >7.

Acrylic polymers (preferred). The performance of acrylic polymers (primarily their solubility in biological fluids) can vary based on the degree and type of substitution. Examples of suitable acrylic polymers include methacrylic acid copolymers and ammonio 10 methacrylate copolymers. The Eudragit series E, L, S, RL, RS and NE (Rohm Pharma) are available as solubilized in organic solvent, aqueous dispersion, or dry powders. The Eudragit series RL ,NE, and RS are insoluble in the gastrointestinal tract but are permeable and are used primarily for extended release. The Eudragit series E dissolve in the stomach. The Eudragit series L, L-30D and S are insoluble in stomach and dissolve in 15 the intestine.

Cellulose Derivatives (also preferred). Examples of suitable cellulose derivatives are:

ethyl cellulose;

reaction mixtures of partial acetate esters of cellulose with phthalic 20 anhydride. The performance can vary based on the degree and type of substitution. Cellulose acetate phthalate (CAP) dissolves in pH > 6. Aquateric (FMC) is an aqueous based system and is a spray dried CAP psuedolatex with particles < 1 μ m. Other components in Aquateric can include pluronic, Tweens, and acetylated monoglycerides;

25 cellulose acetate trimellitate (Eastman);
methylcellulose (Pharmacoat, Methocel);
hydroxypropyl methyl cellulose phthalate (HPMCP). The performance can vary based on the degree and type of substitution. HP-50, HP-55, HP-55S, HP-55F grades are suitable;

30 hydroxypropyl methyl cellulose succinate (HPMCS; AQOAT (Shin Etsu)).
The performance can vary based on the degree and type of substitution. Suitable grades include AS-LG (LF), which dissolves at pH 5, AS-MG (MF), which dissolves at

1 pH 5.5, and AS-HG (HF), which dissolves at higher pH. These polymers are offered as granules, or as fine powders for aqueous dispersions;

Poly Vinyl Acetate Phthalate (PVAP). PVAP dissolves in pH >5, and it is much less permeable to water vapor and gastric fluids; and

5 Coteric (by Colorcon).

Combinations of the above materials can also be used.

The coating can, and usually does, contain a plasticizer and possibly other coating excipients such as colorants, talc, and/or magnesium stearate, which are well known in the art. Suitable plasticizers include: triethyl citrate (Citroflex 2), triacetin (glyceryl 10 triacetate), acetyl triethyl citrate (Citroflec A2), Carbowax 400 (polyethylene glycol 400), diethyl phthalate, tributyl citrate, acetylated monoglycerides, glycerol, fatty acid esters, propylene glycol, and dibutyl phthalate. In particular, anionic carboxylic acrylic polymers usually will contain 10-25% by weight of a plasticizer, especially dibutyl phthalate, polyethylene glycol, triethyl citrate and triacetin. Conventional coating techniques such as 15 spray or pan coating are employed to apply coatings. The coating thickness must be sufficient to ensure that the oral dosage form remains intact until the desired site of topical delivery in the lower intestinal tract is reached.

Colorants, detackifiers, surfactants, antifoaming agents, lubricants, stabilizers such as hydroxy propyl cellulose, acid/base may be added to the coatings besides plasticizers to 20 solubilize or disperse the coating material, and to improve coating performance and the coated product.

A particularly suitable methacrylic copolymer is Eudragit L.RTM, particularly L-25 30D.RTM and Eudragit 100-55.RTM, manufactured by Rohm Pharma, Germany. In Eudragit L-30 D.RTM, the ratio of free carboxyl groups to ester groups is approximately 1:1. Further, the copolymer is known to be insoluble in gastrointestinal fluids having pH below 5.5, generally 1.5-5.5, i.e., the pH generally present in the fluid of the upper gastrointestinal tract, but readily soluble or partially soluble at pH above 5.5, i.e., the pH generally present in the fluid of lower gastrointestinal tract.

Another methacrylic acid polymer which is suitable for use in coating the oral 30 dosage forms and/or the granules, particles, pellets or beads of absorption enhancing carrier and/or hydrophilic therapeutic agent which can be employed in the compositions and methods described herein, either alone or in combination with other coatings, is

- 1 Eudragit S.RTM, manufactured by Rohm Pharma, Germany. Eudragit S.RTM. differs
from Eudragit L-30-D.RTM only insofar as the ratio of free carboxyl groups to ester
groups is approximately 1:2. Eudragit S.RTM is insoluble at pH below 5.5, but unlike
Eudragit L-30-D.RTM, is poorly soluble in gastrointestinal fluids having pH of 5.5-7.0,
5 such as is present in the small intestine media. This copolymer is soluble at pH 7.0 and
above, i.e., the pH generally found in the colon. Eudragit S.RTM can be used alone as a
coating to provide delivery of the hydrophilic therapeutic agent and/or the absorption
enhancing carrier beginning at the large intestine via a delayed release mechanism. In
addition, Eudragit S.RTM, being poorly soluble in intestinal fluids below pH 7, can be
10 used in combination with Eudragit L-30-D.RTM, soluble in intestinal fluids above pH 5.5,
in order to effect a delayed release composition which can be formulated to deliver the
hydrophilic therapeutic agent and/or absorption enhancing carrier to various segments of
the intestinal tract. The more Eudragit L-30 D.RTM used the more proximal realease and
delivery begins, and the more Eudragit S.RTM used, the more distal release and delivery
begins. Both Eudragit L-30-D.RTM and Eudragit S.RTM can be substituted with other
15 pharmaceutically acceptable polymers with similar pH solubility characteristics.

Preferred materials include shellac, acrylic polymers, cellulosic derivatives,
polyvinyl acetate phthalate, and mixtures thereof. More preferred materials include
20 Eudragit series E, L, S, RL, RS, NE, L.RTM, L300.RTM, S.RTM, 100-55RTM, cellulose
acetate phthalate, Aquateric, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl
methyl cellulose phthalate, hydroxypropyl methyl cellulose succinate, poly vinyl acetate
phthalate, and Cotteric. Most preferred materials include Eudragit series L.RTM,
L300.RTM, S.RTM, L100-55RTM, cellulose acetate phthalate, Aquateric, ethyl cellulose,
25 hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose succinate, poly
vinyl acetate phthalate, and Cotteric.

Extended release and targeted delayed release coatings for dosage forms of the
compositions of the present invention are described more completely in U.S. Patent Nos.
30 5,622,721 and 5,686,105, the disclosures of which are incorporated herein by reference in
their entirety.

Although formulations specifically suited to oral administration are presently
preferred, the compositions of the present invention can also be formulated for topical,
transdermal, buccal, nasal, ocular, pulmonary, vaginal, rectal, transmucosal or parenteral

1 administration, as well as for oral administration. Thus, the dosage form can be a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup, elixer, or other dosage form, as desired. If formulated as a suspension, the composition can further be processed in capsule form.

5 When formulated as a sprayable solution or dispersion, a dosage form of a multiparticulate carrier coated onto a substrate with the pharmaceutical compositions described herein can be used. The substrate can be a granule, a particle, a pellet or a bead, for example, and formed of a therapeutic agent or a pharmaceutically acceptable material. The multiparticulate carrier can be enteric coated with a pharmaceutically acceptable 10 material, such as the targeted delayed-enteric coatings and extended release coatings of U.S. Patent Nos. 5,622,721 and 5,686,105, described above. The multiparticulate carrier, coated or uncoated, can further be processed by encapsulation, and the resultant capsule can also be coated, if desired.

15 Other additives may be included, such as are well-known in the art, to impart the desired consistency and other properties to the formulation.

8. Specific Embodiments

In all of the embodiments described herein, the components of the absorption enhancing carrier are present in amounts such that upon mixing with an aqueous diluent, either *in vitro* or *in vivo*, the carrier forms an aqueous dispersion having a small average 20 particle size. In a preferred embodiment, the dispersion is also substantially optically clear. In these preferred embodiments, the optical clarity or particle size in an aqueous dispersion defines the preferred relative concentrations of the components as described above, but does not restrict the dosage form of the compositions to an aqueous dispersion, nor does it limit the compositions of the invention to optically clear dosage forms. Thus, 25 the preferred concentrations of the components are determined by the particle size and/or optical clarity of a dispersion formed by the composition preconcentrate and an aqueous diluent in a dilution of about 10 to about 250-fold, as a preliminary matter. Once the appropriate concentrations are determined, the pharmaceutical compositions can be formulated as described in the preceding section, without regard to the optical clarity of 30 the ultimate formulation in these preferred embodiments.

In one particular embodiment, the present invention provides a triglyceride-free pharmaceutical system including an absorption enhancing composition including at least

1 two surfactants, at least one of which is hydrophilic. The surfactants are present in
amounts such that the carrier forms an aqueous dispersion having a small average particle
size. In one preferred aspect of this embodiment, the average particle size is less than
about 200 nm upon mixing with an aqueous diluent. In another preferred aspect of this
5 embodiment, the aqueous dispersion is substantially optically clear. Preferably, the
composition includes a mixture of hydrophilic and hydrophobic surfactants.

The pharmaceutical system also includes a hydrophilic therapeutic agent. The hydrophilic therapeutic agent can be solubilized, suspended, or partially solubilized and suspended, in the dosage form of the absorption enhancing composition. Alternatively,
10 the hydrophilic therapeutic agent can be provided in a separate dosage form, so that in use,
the dosage form of the absorption-enhancing composition and the dosage form of the
hydrophilic therapeutic agent are co-administered. In the latter aspect, the pharmaceutical
system can make use of any dosage form of a hydrophilic therapeutic agent, such as
15 commercially available dosage forms. The pharmaceutical system is particularly
advantageous, since the absorption enhancing pharmaceutical composition improves the
functionality of even conventionally formulated hydrophilic therapeutic agents.
Preferably, the dosage form of the absorption enhancing pharmaceutical composition, with
or without a hydrophilic therapeutic agent, is an orally administrable dosage form. If the
20 hydrophilic therapeutic agent is provided in a separate dosage form, it is preferred that the
dosage form of the hydrophilic therapeutic agent also be an orally administrable dosage
form.

In another aspect, the present invention provides a method of improving the
bioabsorption of a hydrophilic therapeutic agent administered to a patient, such as an
animal, preferably a mammal, and more preferably a human. The method includes the
25 steps of providing a dosage form of an absorption enhancing composition, providing a
hydrophilic therapeutic agent, and administering the dosage form of the absorption
enhancing composition and the hydrophilic therapeutic agent to the patient. The dosage
form of the absorption enhancing composition can be any of the dosage forms described
above. Similarly, the hydrophilic therapeutic agent can be provided solubilized,
30 suspended, or partially solubilized and suspended, in the dosage form of the absorption
enhancing composition, or can be provided in a separate dosage form. It is surprisingly
found that by administering a hydrophilic therapeutic agent contained within, or co-

1 administered with, a dosage form of an absorption enhancing composition of the present
invention, the rate and/or extent, or the consistency in the rate and/or extent of
bioabsorption of the hydrophilic therapeutic agent is unexpectedly enhanced. Thus, in one
5 aspect the method increases the rate and/or extent of bioabsorption. In another aspect, the
method increases the consistency of the rate and/or extent of bioabsorption. In this latter
aspect, the rate and/or extent of bioabsorption can be greater than or less than the rate that
would be seen using conventional methods.

10 In other embodiments, the absorption enhancing compositions in the
pharmaceutical systems and methods of the present invention can be free of water in the
preconcentrate form, free of propylene glycol diesters, and/or free of cholesterol. All of
the compositions, however, are substantially free of triglycerides.

9. Preparation of Pharmaceutical Compositions

15 The pharmaceutical compositions of the present invention can be prepared by
conventional methods well known to those skilled in the art. Of course, the specific
method of preparation will depend upon the ultimate dosage form. For dosage forms
substantially free of water, *i.e.*, when the composition is provided in a pre-concentrate
form for later dispersion *in vitro* or *in vivo* in an aqueous system, the composition is
prepared by simple mixing of the components to form a pre-concentrate. The mixing
process can be aided by gentle heating, if desired. For compositions in the form of an
20 aqueous dispersion, the pre-concentrate form is prepared, then the appropriate amount of
an aqueous diluent is added. Upon gentle mixing, an aqueous dispersion is formed. If any
water-soluble enzyme inhibitors or additives are included, these may be added first as part
of the pre-concentrate, or added later to the aqueous dispersion, as desired. The dosage
forms of the absorption enhancing compositions can be prepared with or without a
25 hydrophilic therapeutic agent, and a hydrophilic therapeutic agent may also be provided in
the diluent, if desired, or in a separate dosage form.

As previously noted, in another embodiment, the present invention includes a
multi-phase dispersion containing a hydrophilic therapeutic agent. In this embodiment, a
30 dosage form includes a hydrophilic therapeutic agent and an absorption enhancing
composition which forms an aqueous dispersion upon mixing with an aqueous diluent, and
an additional amount of non-solubilized hydrophilic therapeutic agent. Thus, the term
“multi-phase” as used herein to describe these compositions of the present invention

1 means a composition which when mixed with an aqueous diluent forms an aqueous phase
and a particulate dispersion phase. The composition components are as described above,
and can include any of the surfactants, therapeutic agents, solubilizers and additives
previously described. An additional amount of hydrophilic therapeutic agent is included
5 in the composition. This additional amount is not solubilized in the composition, and
upon mixing with an aqueous system is present as a separate dispersion phase. The
additional amount is optionally a milled, micronized, or precipitated form. Thus, upon
dilution, the composition contains two phases: an aqueous dispersion phase containing a
first, solubilized amount of the hydrophilic therapeutic agent, and a second, non-
10 solubilized amount of the hydrophilic therapeutic agent dispersed therein.

One skilled in the art will appreciate that a hydrophilic therapeutic agent may have
a greater solubility in the pre-concentrate composition than in the aqueous dispersion, so
that meta-stable, supersaturated solutions having apparent optical clarity but containing a
15 hydrophilic therapeutic agent in an amount in excess of its solubility in the aqueous
dispersion can be formed. Such super-saturated solutions, whether characterized as
aqueous dispersions (as initially formed) or as multi-phase solutions (as would be
expected if the meta-stable state breaks down), are also within the scope of the present
invention.

The multi-phase formulation can be prepared by the methods described above. A
20 pre-concentrate is prepared by simple mixing of the components, with the aid of gentle
heating, if desired. It is convenient to consider the hydrophilic therapeutic agent as
divided into two portions, a first solubilizable portion which will be solubilized and
contained within the clear aqueous dispersion upon dilution, and a second non-
solubilizable portion which will remain non-solubilized. When the ultimate dosage form
25 is non-aqueous, the first and second portions of the hydrophilic therapeutic agent are both
included in the pre-concentrate mixture. When the ultimate dosage form is aqueous, the
composition can be prepared in the same manner, and upon dilution in an aqueous system,
the composition will form the two phases as described above, with the second non-
solubilizable portion of the hydrophilic therapeutic agent dispersed or suspended in the
30 aqueous system, and the first solubilizable portion of the hydrophilic therapeutic agent
solubilized in the composition. Alternatively, when the ultimate dosage form is aqueous,
the pre-concentrate can be prepared including only the first, solubilizable portion of the

1 hydrophilic therapeutic agent. This pre-concentrate can then be diluted in an aqueous system to form an aqueous dispersion, to which is then added the second, non-solubilizable portion of the hydrophilic therapeutic agent to form a multi-phase aqueous composition.

5 **B. Characteristics of the Pharmaceutical Compositions and Methods**

The dispersions formed upon dilution of the pharmaceutical compositions of the present invention are believed to have some or all of the following characteristics:

10 **Rapid formation:** upon dilution with an aqueous diluent, the composition forms an aqueous dispersion of small particle size very rapidly; *i.e.*, the dispersion appears to form instantaneously.

15 **Optical clarity:** in a preferred embodiment, the dispersions are essentially optically clear to the naked eye, and show no readily observable signs of heterogeneity, such as turbidity or cloudiness. More quantitatively, dispersions of the pharmaceutical compositions of the present invention have absorbances (400 nm) of less than about 0.3, and generally less than about 0.1, at 100X dilution in this preferred embodiment. In the multi-phase embodiment of the compositions described herein, it should be appreciated that the optical clarity of the aqueous phase will be obscured by the dispersed particulate non-solubilized hydrophilic therapeutic agent.

20 **Small Particle Size:** dispersions of the pharmaceutical compositions of the present invention contain particles of very small size. Preferably, the average size is less than about 200 nm, more preferably less than about 100 nm, still more preferably less than about 50 nm and most preferably less than about 20 nm. The small particle size promotes efficient transport of the absorption enhancing components to the absorption site.

25 **Robustness to dilution:** the dispersions are surprisingly stable to dilution in aqueous solution. The absorption enhancing composition remains solubilized for at least the period of time relevant for absorption.

The unique pharmaceutical compositions and methods of the present invention present a number of significant and unexpected advantages, including:

30 **Efficient transport:** The particle sizes in the aqueous dispersions of the present invention are much smaller than the larger particles characteristic of vesicular, emulsion or microemulsion phases. This reduced particle size enables more efficient transport through the intestinal aqueous boundary layer, and through the absorptive brush border membrane.

- 1 More efficient transport to absorptive sites leads to improved and more consistent absorption of therapeutic agents. Moreover, the present invention allows absorption enhancing components to be delivered to the absorption site along with the hydrophilic therapeutic agent, to further enhance absorption.
- 5 No dependence on lipolysis: The lack of triglycerides provides pharmaceutical compositions that are not dependent upon lipolysis, and upon the many poorly characterized factors which affect the rate and extent of lipolysis, for effective presentation of a therapeutic agent to an absorptive site. Such factors include the presence of composition components which may inhibit lipolysis; patient conditions which limit
- 10 production of lipase, such as pancreatic lipase secretory diseases; and dependence of lipolysis on stomach pH, endogenous calcium concentration, and presence of co-lipase or other digestion enzymes. The lack of lipolysis dependence further provides transport which is less prone to suffer from any lag time between administration and absorption caused by the lipolysis process, enabling a more rapid onset of therapeutic action and
- 15 better bioperformance characteristics. In addition, pharmaceutical compositions of the present invention can make use of hydrophilic surfactants which might otherwise be avoided or limited due to their potential lipolysis inhibiting effects.

20 Non-dependence on bile and meal fat contents: Due to the higher solubilization potential over bile salt micelles, the present compositions are less dependent on endogenous bile and bile related patient disease states, and meal fat contents. These advantages overcome meal-dependent absorption problems caused by poor patient compliance with meal-dosage restrictions.

25 Faster dissolution and release: Due to the robustness of compositions of the present invention to dilution, the components of the absorption enhancing composition remain solubilized and thus do not suffer problems of precipitation or agglomeration in the time frame relevant for absorption. In addition, the therapeutic agent is presented in small particle carriers, and is not limited in dilution rate by entrapment in emulsion carriers.

30 Consistent performance: Aqueous dispersions of the present invention are thermodynamically stable for the time period relevant for absorption, and can be more predictably reproduced, thereby limiting variability in bioavailability-- a particularly important advantage for therapeutic agents with a narrow therapeutic index.

1 Less prone to gastric emptying delays: Unlike conventional triglyceride-containing formulations, the present compositions are less prone to gastric emptying delays, resulting in faster absorption. Further, the particles in dispersions of the present invention are less prone to unwanted retention in the gastro-intestinal tract.

5 Better targeted absorption: The compositions of the present invention can be targeted to specific absorption sites through targeted enteric coating or extended release coating, thus minimizing dilution effects and optimizing activity of the hydrophilic therapeutic agent.

10 These and other advantages of the present invention, as well as aspects of preferred embodiments, are illustrated more fully in the Examples which follow.

EXAMPLES

Example 1: Preparation of Compositions

A simple pre-concentrate is prepared as follows. Predetermined weighed amounts of the components are stirred together to form a homogeneous mixture. For combinations 15 that are poorly miscible, the mixture can be gently heated to aid in formation of the homogeneous mixture. If the composition is to include a hydrophilic therapeutic agent, the chosen hydrophilic therapeutic agent in a predetermined amount can be added and stirred until solubilized. Optionally, solubilizers or additives are included by simple mixing.

20 To form an aqueous dispersion of the pre-concentrate, a predetermined amount of an aqueous medium such as purified water, buffer solution, or aqueous simulated physiological solution, is added to the pre-concentrate, and the resultant mixture is stirred to form an aqueous dispersion. Of course, when the dosage form is an aqueous dispersion, any of the components that are readily water-soluble, including the hydrophilic therapeutic 25 agent, can be provided in the diluent solution.

Examples 2-3: Membrane Transport and In Situ Absorption Studies

Compositions of the present invention were tested by two different methods, to demonstrate the improved delivery of hydrophilic therapeutic agents incorporated within or co-administered with compositions including an absorption enhancing carrier. In one 30 set of studies, the relative permeability of membranes to hydrophilic therapeutic agents was compared with and without the presence of an absorption enhancing carrier ("Membrane Transport Study"). In a second set of studies, the relative absorption of a

- 1 hydrophilic therapeutic agent in rat mesenteric veins was compared with and without the
presence of an absorption enhancing carrier ("Relative Absorption Study").

5 For Examples 2 and 3, the following compositions were used, as described in the following sections. For each sample composition, absorbance measurements were made at 400 nm, using a UV-Visible spectrophotometer, at a dilution of 25X with distilled water. In addition, particle size measurements were made using a particle size analyzer, and the volume-weighted average particle sizes are shown along with sample characteristics in Table 19. The standard deviation of the particle size distribution is shown in parentheses next to the average particle size.

10

Table 19: Sample Compositions and Characterizations

| Sample No. | Components | Amounts (g) | Absorbance | Size (nm) |
|------------|---------------------|-------------|------------|------------|
| 15 | Cremophor RH40 | 0.50 | 0.016 | 14.1 (2.5) |
| | Labrasol | 0.20 | | |
| | Capmul MCM | 0.30 | | |
| 20 | Tween 20 | 0.67 | 0.039 | 12.3 (2.1) |
| | Lauroglycol | 0.16 | | |
| | Glycofufrol | 0.17 | | |
| 25 | Cremophor RH40 | 0.30 | 0.004 | 9.0 (1.6) |
| | Arlacel 186 | 0.20 | | |
| | Sodium taurocholate | 0.18 | | |
| | Propylene glycol | 0.32 | | |
| 30 | Cremophor RH40 | 0.54 | 0.167 | 17.6 (3.8) |
| | Span 80 | 0.26 | | |
| | PEG 400 | 0.20 | | |
| 35 | Cremophor RH40 | 0.06 | 2.497 | 2610 (564) |
| | Arlacel 186 | 0.62 | | |
| | Propylene glycol | 0.32 | | |
| 40 | Cremophor RH40 | 0.49 | -0.010 | 13.8 (2.3) |
| | Propylene glycol | 0.51 | | |

Note that Sample Nos. 5 and 6 are control samples. Sample No. 5 was observed to form a cloudy emulsion upon mixing with an aqueous diluent, and fails to show a small particle size. Sample No. 6 contains only one surfactant.

1

Example 2: Membrane Transport StudiesExperimental

The membrane transport studies of model hydrophobic therapeutic agents were carried out across the CACO-2 monolayers. The Caco-2 cell line, originating from a human carcinoma, was obtained from the American Type Culture collection and was grown to form confluent monolayers as described elsewhere (I.J. Hidalgo, T.J. Raub, and R.T. Borchardt, *Gastroenterology* 96:736-749 (1989)). All cells used in this study were between 50 and 60 passage number. The cells were measured for confluency by measurement of TEER (trans epithelial electrical resistance) values. Monolayers exhibiting similar TEER values consistent with "non leakiness" were used to study and compare transport characteristics of model actives in plain buffer and in presence of diluted compositions of the present invention.

In duplicate, all transport experiments were performed for 2 hrs at 37°C in pH 7.35 HBSS containing 25 mM glucose and 10 mM Hepes buffer. Prior to the experiments, the culture medium of Transwell grown Caco-2 cell monolayers was replaced with transport medium equilibrated at 37°C, and the cell monolayer was subsequently equilibrated before undertaking transport studies.

Two hydrophilic therapeutic agents, foscarnet and PEG-4000, were tested. Foscarnet sodium is a low molecular weight (192 g/mol) hydrophilic antiviral that inhibits viral DNA polymerase and reverse transcriptase. It is very soluble in water, shows pK_as of 0.5, 3.4 and 7.3, and has a log of octanol/water partition coefficient of -2.0 (at pH 7.4). Apical to basal transport of the model hydrophilic actives foscarnet sodium and polyethylene glycol 4000 (PEG-4000) was studied by spiking the transport medium, a plain buffer or a 100X buffer dilution of the composition under investigation, with one micro curie of radio-labeled active on the apical side. Basolateral appearance of the active was monitored by taking appropriate samples and assaying for radioactivity. Permeability coefficients (P) were calculated using the following equation:

$$P = (dQ/dt) / (AC_0)$$

where P is the permeability coefficient, dQ/dt is the flux across the monolayer (DPM/min), A is the surface area of the membrane, and C₀ is the initial concentration of the active.

1 Results:

Table 20 shows the apical to basal membrane transport of a conventional hydrophilic active, foscarnet sodium in Sample Nos. 1-3, and a model macromolecular hydrophilic active, PEG-4000, in Sample No. 4, compared to a plain buffer solution

5

Table 20: Permeability for a Conventional Hydrophilic Active

| Sample No. | Active | $(P_{sample}^a / P_{buffer}^b) \times 100$ |
|------------|------------------|--|
| 1 | foscarnet sodium | 1007 |
| 2 | foscarnet sodium | 195 |
| 3 | foscarnet sodium | 160 |
| 4 | PEG-4000 | 188 |

^a permeability in the presence of 100X diluted composition

15

^b permeability in the presence of buffer only

Example 3: Relative Absorption Study

Experimental:

The sample preconcentrate solutions were diluted with standard hypotonic PBS pH 7.4 buffer. Two hydrophilic therapeutic agents were studied: a conventional hydrophilic active, acyclovir, and the model macromolecular active, PEG-4000.

For the acyclovir compositions, the compositions after dilution were spiked with 0.1 mM cold acyclovir, then 0.5 microliter of tritiated acyclovir (specific activity 18.9 Ci/mmol) was added to the diluted composition. The osmotic pressure was adjusted with sodium chloride as needed. The resulting aqueous isotonic dispersions were perfused through rat intestinal segments to assess absorption enhancement in a procedure described below. Appearance of the active was monitored in the mesenteric blood along with disappearance on the luminal side.

Surprisingly, appreciable levels of the conventional hydrophilic active were noted in the blood compared to control perfusion studies conducted with plain buffer and with the control samples 5 (milky emulsion-forming preconcentrate) and 6 (plain one surfactant concentrate), showing that the compositions of the present invention increased absorption characteristics of very hydrophilic actives.

1 For the model macromolecular active, radio labeled PEG-4000 was added to a
diluted (50X) pre-concentrate, and the resulting clear aqueous isotonic dispersion was
perfused through a rat intestinal segment to assess absorption enhancement in a procedure
described below. Appearance of the active was monitored in the mesenteric blood along
5 with disappearance on the luminal side. Surprisingly, as with the acyclovir, appreciable
levels of hydrophilic active were noted in the blood compared to control perfusion studies
conducted with plain buffer, showing the unexpected result that the compositions of the
present invention increased permeability characteristics of very hydrophilic
macromolecular actives.

10 Procedure:

Young adult (275-300 g) male Sprague Dawley rats were used. The procedures
were consistent with those reported by Winne et al, "In vivo studies of mucosal-serosal
transfer in rat jejunum", *Naunyn-Schmeideberg's Arch. Pharmacol.*, 329, 70 (1985).

15 Jugular vein cannulation: the animal was anesthetized using 2% halothane in 98%
oxygen via a halothane vaporizer (Vapomatic, A.M. Bickford, Inc., NY). An opening in
the jugular vein was made with a 21 gauge needle and a jugular cannula consisting of a 4
cm segment of silastic tubing connected to polyethylene tubing was inserted in the jugular
vein and secured with cyanoacrylate glue. For the donor rat, approximately 20 mL of
20 blood was freshly collected in the presence of heparin (1,000 units) and the collected
blood was infused at a rate of 0.2 mL/min through the jugular vein in the experimental rat
to replenish blood sampling.

25 Intestine cannulation: after the animal was anesthetized, its body temperature was
maintained at 37 °C using a heating pad. A vertical midline incision of approximately 3
cm was made through the skin to expose the small intestine. Approximately 6-10 cm
segment of ileum was located. Using electro-cautery, a small incision was made at the
ends of the segment and the luminal contents were flushed with saline maintained at 37
°C. Two 1.5 cm notched pieces of Teflon tubing were inserted into the intestinal lumen at
each incision and tightened using 4-0 silk. A warm isotonic buffer was passed through the
intestine using a 50-mL syringe. These teflon cannula were used to perfuse the drug
30 solution through the isolated intestinal segment using a syringe pump.

Mesenteric vein cannulation: the mesenteric vein draining blood from the resulting
isolated mesenteric cascade venule was then cannulated using a 24 gauge IV catheter and

1 secured in place using 4-0 silk sutures. The cannula was then connected to a polyethylene tubing 25 cm long where the blood was collected in a vial kept under the animal level. Blood samples were collected continuously over 60 to 90 min. The infusion of blood via the jugular vein was initiated to replenish blood loss.

5 Results:

I. Conventional Hydrophilic Active (acyclovir)

The experiment was performed twice for each of the test samples and control buffer compositions. For each formulation, the results of the two trials were averaged. The cumulative amount of radioactivity for the duration of the study as a fraction of total 10 radioactivity exposed to the intestinal segment was monitored for each trial to assess absorption. The % relative absorption results for a conventional hydrophilic active (acyclovir) in presence of various diluted example compositions compared to a plain buffer are presented in Table 21. The relative absorption reported in Table 21 is 100 times the ratio of the fraction of the total amount administered in mesenteric blood when 15 perfused with the 25X diluted compositions to the fraction of the total amount administered when perfused with the plain buffer, over the same time period.

Table 21: Relative Absorption of Acyclovir

| Sample No. | % Relative Absorption |
|------------------|-----------------------|
| 1 | 614 |
| 2 | 634 |
| 3 | 704 |
| Control Samples: | |
| 5 | 171 |
| 6 | 141 |

Surprisingly, appreciable bioenhancement was observed only for compositions that had at least one hydrophilic surfactant plus a second surfactant, and that formed very small dispersions upon dilution (Sample Nos. 1-3), showing that effective presentation of carrier 30 at the absorption site is very critical. In contrast, compositions that contained the same surfactants but formed larger unstable emulsion upon dilution (Sample No. 5) due to poor choice of concentration, or contained only a single surfactant (Sample No. 6) resulted in only marginal bioenhancement over plain buffer.

1 II. Macromolecular Hydrophilic Active

The results for a macromolecular hydrophilic active is presented in Table 22. The experiment was performed twice for each composition. The relative absorption shown in the Table is for a 50X dilution

5

Table 22: Relative Absorption of a Macromolecular Active

| Sample No. | % Relative Absorption |
|------------|-----------------------|
| 3 | 991 |

In comparison to negligible absorption of PEG 4000 in presence of plain buffer, 10 the absorption of PEG 4000 in the presence of a composition of the present invention gave surprising high absorption. This demonstrates the improved absorption of macromolecules with compositions of the present invention.

15 Typical surfactant ratios consistent with the invention that can be prepared are listed. Additives can be included as discussed herein, and the concentrations can be varied as desired to render the compositions easy to prepare, stable upon storage, bioacceptable and elegant, provided that the concentrations are such that the carrier forms an aqueous dispersion having a small particle size, upon dilution with an aqueous medium. Adequate enzyme inhibitor, bufferants, other additives and organic solubilizers can be included at 20 pharmaceutically acceptable levels. Hydrophilic therapeutic agents can be added at levels convenient for therapeutic effect.

20 A: Compositions Having At least Two Hydrophilic Surfactants

| | |
|--------------------------|-------|
| Sodium taurocholate | 0.18g |
| Cremophor RH 40 | 0.30g |
| Sodium chenodeoxycholate | 0.30g |
| Tween 80 | 0.50g |
| Sodium Sarcocholate | 0.15g |
| Crovol M-70 | 0.60g |
| Sodium lithocholate | 0.30g |
| Labrasol | 0.55g |
| Sodium glycocholate | 0.10g |
| Tween 20 | 0.50g |

| | | |
|----|--|----------------------------------|
| 1 | Sodium ursodeoxycholate Incrocas-35 | 0.30 0.50 |
| | Chenodeoxycholic acid Cremophor RH 40 | 0.25g 0.50g |
| 5 | Cremophor RH 40 Sodium caprate | 0.60g 0.10g |
| | Cremophor RH 40 Palmitoyl carnitine | 0.50g 0.20g |
| 10 | Solulan C-24 Sodium chenodeoxycholate | 0.60g 0.25g |
| | Taurocholate Egg or Soy lecithin | 0.20g 0.09g |
| | Tween 20 Sodium taurocholate | 0.30g 0.20g |
| 15 | Tween 20 Egg lecithin | 0.25g 0.15g |
| | Chenodeoxycholate C_{18} lysolipid | 0.18g 0.10g |
| 20 | Chenodeoxycholate Oleic acid Labrasol Brij 35 | 0.20g 0.10g 0.20g 0.75g |

B: Compositions Having One Hydrophilic and One Hydrophobic Surfactant

| | | |
|----|--|----------------|
| 25 | Cremophor EL-P Peceol | 0.83g 0.17g |
| | Cremophor EL-P Propylene glycol monocaprate | 0.50g 0.20g |
| 30 | Cremophor EL-P Imwitor 375 | 0.50g 0.20g |
| | Cremophor EL-P Nikkol MGM | 0.50g 0.18g |
| | Cremophor RH 40 | 0.50g |

| | | |
|----|-----------------------------|-------|
| 1 | Arlacel 186 | 0.10g |
| | Cremophor RH 40 | 1.53g |
| | Arlacel 186 | 0.38 |
| | HPB cyclodextrin | 0.18g |
| 5 | Cremophor RH 40 | 0.55g |
| | Capmul MCM | 0.80g |
| | Cremophor RH 40 | 0.50g |
| | Crodamol (ethyl oleate) | 0.28g |
| 10 | Cremophor RH 40 | 0.50g |
| | Labrafil | 0.40g |
| | Cremophor RH 40 | 0.22g |
| | Lauroglycol FCC | 0.20g |
| | Cremophor RH40 | 0.60g |
| | Glyceryl monolaurate | 0.20g |
| 15 | Cremophor RH-40 | 0.43g |
| | Myvacet 9-45 | 0.31g |
| | Cremophor RH-40 | 0.30g |
| | Peccol | 0.11g |
| 20 | Cremophor RH40 | 0.50g |
| | Propyleneglycol monololeate | 0.20g |
| | Cremophor RH40 | 0.50g |
| | Softigen 701 | 0.10g |
| | Cremophor RH40 | 0.50g |
| | Sorbitan monocaprate | 0.25g |
| 25 | Cremophor RH 60 | 0.54g |
| | Span 80 | 0.26g |
| | Cremophor RH 40 | 0.70g |
| | Volpo 3 | 0.30g |
| 30 | Crodet O40 | 0.68g |
| | Plurol Oleique | 0.32g |
| | Crovil M-70 | 0.61g |
| | Crovil M-40 | 0.12g |

| | | |
|----|------------------------------------|----------------|
| 1 | Crovol M-70 Labrafil | 0.38g 0.60g |
| | Crovol M-70 Imwitor 988 | 0.65g 0.15g |
| 5 | Crovol M-70 Linoleic acid | 0.60g 0.20g |
| | Emalex C-40 Gelucire 33/01 | 0.50g 0.15g |
| 10 | Glycerox L Myvacet 9-45 | 0.73g 0.27g |
| | Incrocas 35 Arlacel 186 | 0.65g 0.12g |
| | Incrocas 35 Gelucire 44/14 | 0.25g 0.15g |
| 15 | Incrocas 35 Imwitor 988 | 0.83g 0.20g |
| | Incrocas 35 Labrafil | 0.31g 0.11g |
| 20 | Labrasol Lauroglycol | 0.83g 0.17g |
| | Lauroyl carnitine Imwitor 312 | 0.15g 0.15g |
| | Incrocas 35 Myvacet 9-45 | 0.50g 0.38g |
| 25 | Incrocas-35 Span-20 | 0.50g 0.15g |
| | Incrocas 35 Imwitor 988 | 0.51g 0.22g |
| 30 | Kessco PEG 300DL Gelucire 50/15 | 0.35g 0.50g |
| | Kessco PEG 1540DO Span 80 | 0.65g 0.12 |

| | | |
|----|------------------------------------|----------------|
| 1 | Labrasol Span-20 | 0.45g 0.25g |
| | Myrij 45 Sorbitan monocaprylate | 0.50g 0.25g |
| 5 | Myrij 52 Imwitor 308 | 0.50g 0.20g |
| | Sucrose monolaurate Capmul MCM | 0.50g 0.20g |
| 10 | Nikkol Decaglyn 1-L Crovol M-40 | 0.55g 0.33g |
| | Nikkol Decaglyn 1-0 Capmul MCM | 0.65g 0.25g |
| | Nikkol DHC Nikkol TMGO-5 | 0.67g 0.17g |
| 15 | Nikkol BPS-30 PEG-6 castor oil | 0.30g 0.15g |
| | Tween 20 Drewpol 6-1-0 | 0.75g 0.15g |
| 20 | Tween 20 Lauroglycol FCC | 0.34g 0.11g |
| | Tween 20 Plurol Oleique | 0.58g 0.21g |
| | Tween 80 Lauroglycol | 0.67g 0.17g |
| 25 | Tagat O2 PGMG-03 | 0.50g 0.05g |
| | Tagat L2 Brij 30 | 0.68g 0.32g |
| 30 | Poloxamer 188 Labrafil M2125CS | 0.85g 0.15g |
| | Poloxamer 108 Capmul GMO-K | 0.85g 0.15g |

1 Solulan C-24 0.58g
 Lauroglycol FCC 0.21g

C: Two Hydrophilic Surfactants and One Hydrophobic Surfactant

5 Cremophor EL 0.30g
 Labrasol 0.30g
 Capmul MCM 0.40g

10 Cremophor RH-40 0.25g
 Labrasol 0.25g
 Capmul GMO-K 0.11g

15 Cremophor RH 40 0.30g
 Tween-20 0.20g
 Nikkol Decaglyn 3-O 0.50g

20 Cremophor EL-P 0.45g
 Corvol M-40 0.25g
 Sodium Docusate 0.15g

25 Cremophor RH 40 0.65g
 Arlacel 186 0.15g
 Sodium dodecyl sulfate 0.10g

30 Cremophor RH 40 0.50g
 Peceol 0.20g
 Sodium docusate 0.20g

 Sodium Chenodeoxycholate 0.30g
 Cremophor RH 40 0.40g
 Arlacel 186 0.30g

 Cremophor RH 40 0.41g
 Sodium taurocholate 0.26g
 Arlacel 186 0.27g

 Cremophor RH 40 0.50g
 Softigen 767 0.22g
 Arlacel 186 0.15 g

 Cremophor RH 40 0.40g
 Arlacel 186 0.40g
 Tween 20 0.20g

| | | |
|----|--|-------------------------|
| 1 | Cremophor RH 40 Capmul MCM Sodium chenodeoxycholate | 0.35g 0.30g 0.30g |
| 5 | Kessco PEG 1000MO Labrasol Span 20 | 0.30g 0.30g 0.40g |
| | Polaxamer 188 Pecool Sodium dodecyl sulfate | 0.65g 0.15g 0.10g |
| 10 | Sodium taurocholate Tween 20 Arlacel 186 | 0.17g 0.66g 0.17g |
| | Sodium taurocholate Kessco PEG 1000MO Plurol Oleique | 0.17g 0.66g 0.17g |
| 15 | Sodium taurocholate Tween 80 Arlacel 186 | 0.15g 0.18g 0.18g |
| | Taurochenodeoxycholate Tween 20 Arlacel 186 | 0.15g 0.40g 0.15g |
| 20 | Chenodeoxycholic acid Incrocas-35 Span 20 | 0.25g 0.30g 0.20g |
| | Saurcocholate Cremophor RH 40 Arlacel 186 | 0.20g 0.40g 0.20g |
| 25 | Lithocholate Incrocas-35 Myvacet 9-45 | 0.25g 0.40g 0.30g |
| | Tagat L2 Crovol A-40 Sodium docusate | 0.45g 0.25g 0.15g |
| 30 | Tween -20 Arlacel 186 Sodium chenodeoxycholate | 0.30g 0.20g 0.25g |

| | | |
|---|-----------------|-------|
| 1 | Cremophor RH 40 | 0.40g |
| | Tween-20 | 0.25g |
| | Sodium caprate | 0.25g |
| 5 | Cremophor RH40 | 0.40g |
| | Lauric acid | 0.20g |
| | Incrocas-35 | 0.30g |

D: One Hydrophilic and Two Hydrophobic Surfactants

| | | |
|----|-----------------------|-------|
| 10 | Cremophor RH 40 | 0.50g |
| | Labrafil M2125CS | 0.27g |
| | Crovol M-40 | 0.28g |
| 15 | Cremophor RH 40 | 1.53g |
| | Arlacel 186 | 0.38g |
| | Pecool | 0.38g |
| | HPB beta cyclodextrin | 0.38g |
| 20 | Cremophor RH 40 | 0.55g |
| | Labrafil M2125 CS | 0.34g |
| | Span 80 | 0.2g |
| 25 | Cremophor RH 40 | 0.50g |
| | Labrafil M2125 Cs | 0.27g |
| | Crovol M-40 | 0.28g |

E: Two Hydrophilic and Two Hydrophobic Surfactants

| | | |
|----|-----------------|-------|
| 25 | Polaxamer 108 | 0.45g |
| | Span 20 | 0.25g |
| | Sodium docusate | 0.15g |
| 30 | Ethyl oleate | 0.15g |
| | Softigen 767 | 0.45g |
| | Imwitor 742 | 0.25g |
| | Sodium docusate | 0.15g |
| | Ethyl oleate | 0.15g |

Example 5: Compositions with Hydrophilic Therapeutic Agent

Typical compositions having a hydrophilic therapeutic agent can have components and concentrations in the following exemplary, but not limiting ranges, in percent by weight unless otherwise indicated:

| | | |
|---|--------------------------------------|-----------|
| 1 | absorption enhancing composition | 10-100% |
| | enzyme Inhibitor (e.g., aprotinin) | 0-10% |
| | solubilizer (e.g., propylene glycol) | 0-60% |
| | bufferant | 0-50mM |
| 5 | hydrophilic polymer (e.g., HPMC) | 0-20% w/w |
| | other additives | 0-50% |

If formulated as an aqueous dosage form, a typical amount of water would be about 250 mL, or any other convenient amount.

| | | |
|----|---|-------------|
| 10 | Typical hydrophilic therapeutic agents and amounts in mg or IU/mL or G: | |
| | alendronate Sodium | 5-50mg |
| | etidronate disodium | 200-400 mg |
| | pamidronate disodium | 30-90 mg |
| | aztreonam | 20-500 mg |
| 15 | valacyclovir | 250-1000 mg |
| | gancyclovir | 250-500 mg |
| | famcyclovir | 125-200 mg |
| | pericyclovir | 125-1000 mg |
| | pyridostigmine | 60 mg |
| 20 | cromalyn sodium | 0.1-2mg |
| | nedocromil sodium | 0.1-2 mg |
| | metformin hydrochloride | 500-850 mg |
| | acarbose | 50-100 mg |
| | amphotericin B | 50-200 mg |
| 25 | octreotide acetate | 0.1 to 1 mg |
| | cefoxitin sodium | 200-1000 mg |
| | corticotropin: | 25-1000 IU |
| | sodium heparin | 20-5000 IU |
| | desmopressin acetate (DVAP) | 0.1-1mg |
| 30 | vasopressin | 5-100 IU |
| | salmon calcitonin | 500 IU |
| | insulin | 140 IU |
| | erythropoietin | 14,000 mg |

| | | |
|---|----------------------------|----------|
| 1 | porcine somatotropin | 50 mg |
| | recombinant growth hormone | 30 IU |
| | oligonucleotide | 1-500 mg |

5 Of course, the amounts listed are chosen to be therapeutically effective amounts,
but the invention is not limited thereby.

The present invention may be embodied in other specific forms without departing
from its spirit or essential characteristics. The described embodiments are to be
considered in all respects only as illustrative and not restrictive. The scope of the
invention is, therefore, indicated by the appended claims rather than by the foregoing
10 description. All changes which come within the meaning and range of equivalency of the
claims are to be embraced within their scope.

What is claimed is:

15

20

25

30

- 1 1. A pharmaceutical system for enhanced absorption of a hydrophilic therapeutic agent, the system comprising:
- 5 (a) a dosage form of an absorption enhancing composition, the composition comprising at least two surfactants, at least one of which is hydrophilic; and
- 10 (b) a hydrophilic therapeutic agent,
the pharmaceutical system being substantially free of triglycerides.
- 15 2. The pharmaceutical system of claim 1, wherein the hydrophilic surfactant comprises at least one ionized ionizable surfactant;
- 20 3. The pharmaceutical system of claim 2, wherein the ionized ionizable surfactant is the ionized form of a surfactant selected from the group consisting of bile acids and salts, analogues, and derivatives thereof; lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; carnitine fatty acid ester salts; salts of alkylsulfates; salts of fatty acids; sodium docusate; acyl lactylates; mono-,diacetylated tartaric acid esters of mono-,diglycerides; succinylated monoglycerides; citric acid esters of mono-,diglycerides; and mixtures thereof.
- 25 4. The pharmaceutical system of claim 2, wherein the ionized ionizable surfactant is the ionized form of a surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid, lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate, taurodeoxycholate, chenodeoxycholate, glycodeoxycholate, glycochenodeoxycholate, taurochenodeoxycholate, ursodeoxycholate, lithocholate, taouroursodeoxycholate, glycoursodeoxycholate, cholylsarcosine, N-methyl taurocholate, caproate, caprylate, caprate, laurate, myristate, palmitate, oleate, ricinoleate, linoleate, linolenate, stearate, lauryl sulfate, tetraacetyl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and salts and mixtures thereof.

1 5. The pharmaceutical system of claim 2, wherein the ionized ionizable surfactant is the ionized form of a surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated 5 tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate, chenodeoxycholate, lithocholate, ursodeoxycholate, taurodeoxycholate, glycodeoxycholate, cholylsarcosine, caproate, caprylate, caprate, laurate, oleate, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl 10 carnitine, myristoyl carnitine, and salts and mixtures thereof.

15 6. The pharmaceutical system of claim 2, wherein the ionized ionizable surfactant is the ionized form of a surfactant selected from the group consisting of lecithin, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, chenodeoxycholate, lithocholate, 15 ursodeoxycholate, taurocholate, caprylate, caprate, oleate, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and salts and mixtures thereof.

20 7. The pharmaceutical system of claim 1, wherein the hydrophilic surfactant comprises at least one non-ionic hydrophilic surfactant having an HLB value greater than or equal to about 10.

25 8. The pharmaceutical system of claim 7, wherein the non-ionic surfactant is selected from the group consisting of alkylglucosides; alkylmaltosides; alkylthioglucosides; lauryl macroglycerides; polyoxyethylene alkyl ethers; polyoxyethylene alkylphenols; polyethylene glycol fatty acids esters; polyethylene glycol 30 glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene sterols, derivatives, and analogues thereof; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; sugar esters, sugar ethers; sucroglycerides; and mixtures thereof.

1 9. The pharmaceutical system of claim 7, wherein the non-ionic hydrophilic surfactant is selected from the group consisting of polyoxyethylene alkylethers; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block
5 5 copolymers; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

10 10. The pharmaceutical system of claim 9, wherein the glyceride is a monoglyceride, diglyceride, triglyceride, or a mixture thereof.

11. The pharmaceutical system of claim 9, wherein the reaction mixture comprises the transesterification products of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

15 12. The pharmaceutical system of claim 9, wherein the polyol is glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, or a mixture thereof.

13. The pharmaceutical system of claim 7, wherein the hydrophilic surfactant is PEG-10 laurate, PEG-12 laurate, PEG-20 laurate, PEG-32 laurate, PEG-32 dilaurate, PEG-12 oleate, PEG-15 oleate, PEG-20 oleate, PEG-20 dioleate, PEG-32 oleate, PEG-200 oleate, PEG-400 oleate, PEG-15 stearate, PEG-32 distearate, PEG-40 stearate, PEG-100 stearate, PEG-20 dilaurate, PEG-25 glyceryl trioleate, PEG-32 dioleate, PEG-20 glyceryl laurate, PEG-30 glyceryl laurate, PEG-20 glyceryl stearate, PEG-20 glyceryl oleate, PEG-30 glyceryl oleate, PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-40 palm kernel oil, PEG-50 hydrogenated castor oil, PEG-40 castor oil, PEG-35 castor oil, PEG-60 castor oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-60 corn oil, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polyglyceryl-10 laurate, PEG-30 cholesterol, PEG-25 phytosterol, PEG-30 soya sterol, PEG-20 trioleate, PEG-40 sorbitan oleate, PEG-80 sorbitan laurate, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, POE-20 oleyl ether, POE-20 stearyl ether, tocopheryl PEG-100 succinate, PEG-24 cholesterol, polyglyceryl-10 oleate, Tween 40, Tween 60, sucrose monostearate, sucrose monolaurate, sucrose monopalmitate, PEG

1 10-100 nonyl phenol series, PEG 15-100 octyl phenol series, a poloxamer, or a mixture thereof.

14. The pharmaceutical system of claim 7, wherein the hydrophilic surfactant is PEG-20 laurate, PEG-20 oleate, PEG-35 castor oil, PEG-40 palm kernel oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, polyglyceryl-10 laurate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, PEG-30 cholesterol, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, PEG-24 cholesterol, sucrose monostearate, sucrose monolaurate, a poloxamer, or a mixture thereof.

10 15. The pharmaceutical system of claim 7, wherein the hydrophilic surfactant is PEG-35 castor oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polysorbate 20, polysorbate 80, tocopheryl PEG-1000 succinate, PEG-24 cholesterol, a poloxamer, or a mixture thereof.

15 16. The pharmaceutical system of claim 1, wherein the composition comprises at least two hydrophilic surfactants.

17. The pharmaceutical system of claim 1, wherein the composition comprises at least one hydrophilic surfactant and at least one hydrophobic surfactant.

20 18. The pharmaceutical system of claim 17, wherein the hydrophobic surfactant comprises an un-ionized ionizable surfactant.

19. The pharmaceutical system of claim 18, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of bile acids and analogues and derivatives thereof; lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; carnitine fatty acid esters; alkylsulfates; fatty acids; acyl lactylates; mono-, diacylated tartaric acid esters of mono-, diglycerides; succinylated monoglycerides; citric acid esters of mono-, diglycerides; and mixtures thereof.

25 20. The pharmaceutical system of claim 18, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid,

1 lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine,
lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated
monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid
esters of mono/diglycerides, cholic acid, taurocholic acid, glycocholic acid, deoxycholic
5 acid, taurodeoxycholic acid, chenodeoxycholic acid, glycodeoxycholic acid,
glycochenodeoxycholic acid, taurochenodeoxycholic acid, ursodeoxycholic acid,
lithocholic acid, taurooursodeoxycholic acid, glycoursoodeoxycholic acid, cholylsarcosine,
N-methyl taurocholic acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic
acid, palmitic acid, oleic acid, ricinoleic acid, linoleic acid, linolenic acid, stearic acid,
10 lauryl sulfate, tetraacetyl sulfate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine,
and mixtures thereof.

21. The pharmaceutical system of claim 18, wherein the un-ionized ionizable
surfactant is the un-ionized form of a surfactant selected from the group consisting of
lecithin, lyssolecithin, phosphatidylcholine, phosphatidylethanolamine,
15 phosphatidylglycerol, lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactylic
esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides,
mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of
mono/diglycerides, cholic acid, taurocholic acid, glycocholic acid, deoxycholic acid,
chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid, taurodeoxycholic acid,
20 glycodeoxycholic acid, cholylsarcosine, caproic acid, caprylic acid, capric acid, lauric
acid, oleic acid, lauryl sulfate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine,
and mixtures thereof.

22. The pharmaceutical system of claim 18, wherein the un-ionized ionizable
surfactant is the un-ionized form of a surfactant selected from the group consisting of
lecithin, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated
25 monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid
esters of mono/diglycerides, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic
acid, taurocholic acid, caprylic acid, capric acid, oleic acid, lauryl sulfate, docusate,
lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and mixtures thereof.

30 23. The pharmaceutical system of claim 17 wherein the hydrophobic surfactant
comprises at least one compound having an HLB value less than about 10.

1 24. The pharmaceutical system of claim 23, wherein the hydrophobic
surfactant is selected from the group consisting of alcohols; polyoxyethylene alkylethers;
fatty acids; bile acids; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower
alcohol fatty acids esters; polyethylene glycol fatty acids esters; polyethylene glycol
5 glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene
glycerides; lactic acid derivatives of mono/diglycerides; propylene glycol diglycerides;
sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene
polymer; transesterified vegetable oils; sterols; sterol derivatives; sugar esters; sugar ethers;
10 sucroglycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable
oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids,
glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

15 25. The pharmaceutical system of claim 23, wherein the hydrophobic
surfactant is selected from the group consisting of fatty acids; bile acids; lower alcohol
fatty acid esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty
acid esters; polyoxyethylene glycerides; glycerol fatty acid esters; acetylated glycerol fatty
acid esters; lactic acid derivatives of mono/diglycerides; sorbitan fatty acid esters;
polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block
20 polymers; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable
oils; reaction mixtures of polyols and at least one member of the group consisting of fatty
acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures
thereof.

25 26. The pharmaceutical system of claim 23, wherein the hydrophobic
surfactant is selected from the group consisting of bile acids; lower alcohol fatty acids
esters; polypropylene glycol fatty acid esters; propylene glycol fatty acid esters; glycerol
fatty acid esters; acetylated glycerol fatty acid esters; lactic acid derivatives of
mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene vegetable oils; and mixtures
thereof.

30 27. The pharmaceutical system of claim 23, wherein the hydrophobic
surfactant is a glycerol fatty acid ester, an acetylated glycerol fatty acid ester, or a mixture
thereof.

- 1 28. The pharmaceutical system of claim 27, wherein the glycerol fatty acid ester is a monoglyceride, diglyceride, or a mixture thereof.
- 2 29. The pharmaceutical system of claim 28, wherein the fatty acid of the glycerol fatty acid ester is a C₆ to C₂₂ fatty acid or a mixture thereof.
- 5 30. The pharmaceutical system of claim 23, wherein the hydrophobic surfactant is a reaction mixture of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.
- 10 31. The pharmaceutical system of claim 30, wherein the reaction mixture is a transesterification product of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.
- 15 32. The pharmaceutical system of claim 30, wherein the polyol is polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, or a mixture thereof.
- 20 33. The pharmaceutical system of claim 23, wherein the hydrophobic surfactant is selected from the group consisting of myristic acid; oleic acid; lauric acid; stearic acid; palmitic acid; PEG 1-4 stearate; PEG 2-4 oleate; PEG-4 dilaurate; PEG-4 dioleate; PEG-4 distearate; PEG-6 dioleate; PEG-6 distearate; PEG-8 dioleate; PEG 3-16 castor oil; PEG 5-10 hydrogenated castor oil; PEG 6-20 corn oil; PEG 6-20 almond oil; PEG-6 olive oil; PEG-6 peanut oil; PEG-6 palm kernel oil; PEG-6 hydrogenated palm kernel oil; PEG-4 capric/caprylic triglyceride, mono, di, tri, tetra esters of vegetable oil and sorbitol; pentaerythrityl di, tetra stearate, isostearate, oleate, caprylate, or caprate; polyglyceryl 2-4 oleate, stearate, or isostearate; polyglyceryl 4-10 pentaoleate; polyglyceryl-3 dioleate; polyglyceryl-6 dioleate; polyglyceryl-10 trioleate; polyglyceryl-3 distearate; propylene glycol mono- or diesters of a C₆ to C₂₂ fatty acid; monoglycerides of a C₆ to C₂₂ fatty acid; acetylated monoglycerides of C₆ to C₂₂ fatty acid; diglycerides of C₆ to C₂₂ fatty acids; lactic acid derivatives of monoglycerides; lactic acid derivatives of diglycerides; cholesterol; phytosterol; PEG 5-20 soya sterol; PEG-6 sorbitan tetra, hexastearate; PEG-6 sorbitan tetraoleate; sorbitan monolaurate; sorbitan monopalmitate; sorbitan mono, trioleate; sorbitan mono, tristearate; sorbitan monoisostearate; sorbitan sesquioleate; sorbitan sesquistearate; PEG 2-5 oleyl ether; POE 2-4 lauryl ether; PEG-2 cetyl ether; PEG-2 stearyl ether; sucrose distearate; sucrose dipalmitate; ethyl oleate; isopropyl myristate; isopropyl palmitate; ethyl linoleate; isopropyl linoleate; poloxamers;

1 cholic acid; ursodeoxycholic acid; glycocholic acid; taurocholic acid; lithocholic acid; deoxycholic acid; chenodeoxycholic acid; and mixtures thereof.

34. The pharmaceutical system of claim 23, wherein the hydrophobic surfactant is selected from the group consisting of oleic acid; lauric acid; glyceryl monocaprate; glyceryl monocaprylate; glyceryl monolaurate; glyceryl monooleate; glyceryl dicaprate; glyceryl dicaprylate; glyceryl dilaurate; glyceryl dioleate; acetylated monoglycerides; propylene glycol oleate; propylene glycol laurate; polyglyceryl-3 oleate; polyglyceryl-6 dioleate; PEG-6 corn oil; PEG-20 corn oil; PEG-20 almond oil; sorbitan monooleate; sorbitan monolaurate; POE-4 lauryl ether; POE-3 oleyl ether; ethyl oleate; 10 poloxamers; cholic acid; ursodeoxycholic acid; glycocholic acid; taurocholic acid; lithocholic acid; deoxycholic acid; chenodeoxycholic acid; and mixtures thereof.

35. The pharmaceutical system of claim 1, wherein each of the at least two surfactants is selected from the group consisting of sodium lauryl sulfate, oleic acid, linoleic acid, monoolein, lecithin, lysolecithin, deoxycholate, taurodeoxycholate, 15 glycochenodeoxycholate, polyoxyethylene X-lauryl ether, where X is from 9 to 20, sodium tauro-24,25-dihydrofusidate, polyoxyethylene ether, polyoxyethylene sorbitan esters, p-t-octylphenoxypolyoxyethylene, N-lauryl- β -D-maltopyranoside, 1-dodecylazacycloheptane-2-azone, and phospholipids, and is present in an amount of greater than 10% by weight, based on the total weight of the pharmaceutical system.

20 36. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent is a drug, a vitamin, a nutritional supplement, a cosmeceutical, a diagnostic agent, or a mixture thereof.

37. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent has an apparent water solubility of at least about 1 mg/mL.

25 38. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent is a hydrophilic drug, a cytokine, a peptidomimetic, a peptide, a protein, a toxoid, a serum, an antibody, a vaccine, a nucleoside, a nucleotide, a portion of genetic material, a nucleic acid, or a mixture thereof.

30 39. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent is selected from the hydrophilic members of the group consisting of analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, anti-asthma agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-

- 1 epileptics, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials,
anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents, immunosuppressants,
antiprotozoal agents, anti-thyroid agents, anti-tussives, anxiolytic, sedatives, hypnotics,
neuroleptics, β -Blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-
5 parkinsonian agents, gastro-intestinal agents, histamine H₂-receptor antagonists,
keratolytics, lipid regulating agents, muscle relaxants, anti-anginal agents, nutritional
agents, analgesics, sex hormones, stimulants, cytokines, peptidomimetics, peptides,
proteins, toxoids, sera, antibodies, vaccines, nucleosides, nucleotides, genetic material,
nucleic acids, and mixtures thereof.
- 10 40. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic
agent is selected from the group consisting of acarbose; acyclovir; acetyl cysteine;
acetylcholine chloride; alatrofloxacin; alendronate; algucerase; amantadine
hydrochloride; ambenonium; amifostine; amiloride hydrochloride; aminocaproic acid;
amphotericin B; antihemophilic factor (human); antihemophilic factor (porcine);
15 antihemophilic factor (recombinant); aprotinin; asparaginase; atenolol; atracurium
besylate; atropine; azithromycin; aztreonam; BCG vaccine; bacitracin; becalermin;
belladonna; bepridil hydrochloride; bleomycin sulfate; calcitonin human; calcitonin salmon;
carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium;
20 cefepime hydrochloride; cefixime; cefonicid sodium; cefoperazone; cefotetan disodium;
cefotoxime; cefoxitin sodium; ceftrizoxime; ceftriaxone; cefuroxime axetil; cephalixin;
cephapirin sodium; cholera vaccine; chrionic gonadotropin; cidofovir; cisplatin;
cladribine; clidinium bromide; clindamycin and clindamycin derivatives; ciprofloxacin;
25 clondronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromalyin
sodium; cytarabine; daltaperin sodium; danaproid; deforoxamine; denileukin diftitox;
desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine;
dirithromycin; dopamine hydrochloride; dormase alpha; doxacurium chloride; doxorubicin;
editronate disodium; elanaprilat; enkephalin; enoxacin; enoxaprin sodium; ephedrine;
epinephrine; epoetin alpha; erythromycin; esmol hydrochloride; factor IX; famiciclovir;
fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating
30 factor; granulocyte-macrophage stimulating factor; growth hormones- recombinant
human; growth hormone- bovine; gentamycin; glucagon; glycopyrolate; gonadotropin
releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; grepafloxacin;

- 1 hemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus
vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine;
interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin procine; insulin NPH;
insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta;
5 ipratropium bromide; isofosfamide; japanese encephalitis virus vaccine; lamivudine;
leucovorin calcium; leuprolide acetate; levofloxacin; lincomycin and lincomycin
derivatives; lobucavir; lomefloxacin; loracarbef; mannitol; measles virus vaccine;
meningococcal vaccine; menotropins; mephenzolate bromide; mesalmine; methanamine;
methotrexate; methscopolamine; metformin hydrochloride; metroprolol; mezocillin
10 sodium; mivacurium chloride; mumps viral vaccine; nedocromil sodium; neostigmine
bromide; neostigmine methyl sulfate; neutontin; norfloxacin; octreotide acetate; ofloxacin;
olpadronate; oxytocin; pamidronate disodium; pancuronium bromide; paroxetine;
pefloxacin; pentamidine isethionate; pentostatin; pentoxifylline; periclovir;
15 pentagastrin; phentolamine mesylate; phenylalanine; physostigmine salicylate; plague
vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine
polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymixin B
sulfate; pralidoxine chloride; pramlintide; pregabalin; propofenone; propenthaline
bromide; pyridostigmine bromide; rabies vaccine; residronate; ribavarin; rimantadine
hydrochloride; rotavirus vaccine; salmetrol xinafoate; sincalide; small pox vaccine;
20 solatol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin;
suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiopeta; ticarcillin;
tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; trandolapril;
trimetrexate gluconate; trospectinomycin; trovafloxacin; tubocurarine chloride; tumor
necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valaciclovir; valsartan;
25 varicella virus vaccine live; vasopressin and vasopressin derivatives; vecoronium bromide;
vinblastin; vincristine; vinorelbine; vitamin B12 ; warfarin sodium; yellow fever vaccine;
zalcitabine; zanamavir; zolandronate; and zidovudine.

41. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic
agent is selected from the group consisting of acarbose; acyclovir; atracurium besylate;
30 alendronate; alglucerase; amantadine hydrochloride; amphotericin B; antihemophilic
factor (human); antihemophilic factor (porcine); antihemophilic factor (recombinant;
azithromycin; calcitonin human; calcitonin salmon; capecitabine; cefazolin sodium;

- 1 cefonicid sodium; cefoperazone; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime
axetil; cephalixin; chrionic gonadotropin; cidofovir; cladribine ; clindamycin and
clindamycin derivatives; corticotropin; cosyntropin; cromalyn sodium; cytarabine;
daltaperin sodium; danaproid; desmopressin; didanosine; dirithromycin; editronate
5 disodium; enoxaprin sodium; epoetin alpha; factor IX; famiclovir; fludarabine; foscarnet
sodium; ganciclovir; granulocyte colony stimulating factor; granulocyte-macrophage
stimulating factor; growth hormones- recombinant human; growth hormone- Bovine;
gentamycin; glucagon; gonadotropin releasing hormone and synthetic analogs thereof;
GnRH; gonadorelin; hemophilus B conjugate vaccine; Hepatitis A virus vaccine
10 inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate;
influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin
procine; insulin NPH; insulin aspart; insulin glargine; insulin detemir; interferon alpha;
interferon beta; ipratropium bromide; isofosfamide; lamivudine; leucovorin calcium;
leuprolide acetate; lincomycin and lincomycin derivatives; metformin hydrochloride;
15 nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neutontin;
octreotide acetate; olpadronate; pamidronate disodium; pancuronium bromide;
pentamidine isethionate; pentagastrin; physostigmine salicylate; poliovirus vaccine live
(OPV); pyridostigmine bromide; residronate; ribavarin; rimantadine hydrochloride;
rotavirus vaccine; salmetrol xinafoate; somatostatin; spectinomycin; stavudine;
20 streptokinase; ticarcillin; tiludronate; tissue type plasminogen activator; TNFR:Fc; TNK-
tPA; trimetrexate gluconate; trospectinomycin; tumor necrosis factor; typhoid vaccine
live; urokinase; vancomycin; valaciclovir; vasopressin and vasopressin derivatives;
vinblastin; vincristine; vinorelbine; warfarin sodium; zalcitabine; zanamavir; and
zidovudine.
- 25 42. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic
agent is selected from the group consisting of acarbose; alendronate; amantadine
hydrochloride; azithromycin; calcitonin human; calcitonin salmon; ceftriaxone;
cefuroxime axetil; chrionic gonadotropin; cromalyn sodium; daltaperin sodium;
danaproid; desmopressin; didanosine; editronate disodium; enoxaprin sodium; epoetin
30 alpha; factor IX; famiclovir; foscarnet sodium; ganciclovir; granulocyte colony
stimulating factor; granulocyte-macrophage stimulating factor; growth hormones-
recombinant human; growth hormone- Bovine; glucagon; gonadotropin releasing hormone

1 and synthetic analogs thereof; GnRH; gonadorelin; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin
5 procine interferon alpha; interferon beta; leuprolide acetate; metformin hydrochloride; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neutontin; octreotide acetate; olpadronate; pamidronate disodium; residronate; rimantadine hydrochloride; salmetrol xinafoate; somatostatin; stavudine; ticarcillin; tiludronate; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; tumor necrosis factor; typhoid vaccine live; vancomycin; valacyclovir; vasopressin and vasopressin derivatives; zalcitabine; zanamivir and zidovudine.

10 43. The pharmaceutical system of claim 1, wherein the composition further comprises a solubilizer.

44. The pharmaceutical system of claim 43, wherein the solubilizer is selected from the group consisting of alcohols, polyols, amides, esters, propylene glycol ethers and mixtures thereof.

15 45. The pharmaceutical system of claim 1, wherein the composition further comprises an antioxidant, a bufferant, an antifoaming agent, a detackifier, a preservative, a chelating agent, a viscomodulator, a tonicifier, a flavorant, a colorant, an odorant, an opacifier, a suspending agent, a binder, a filler, a plasticizer, a lubricant, or a mixture thereof.

20 46. The pharmaceutical system of claim 1, wherein the composition further comprises an amount of an enzyme inhibiting agent sufficient to at least partially inhibit enzymatic degradation of the hydrophilic therapeutic agent.

25 47. The pharmaceutical system of claim 46, wherein the enzyme inhibiting agent is P-aminobenzamidine, FK-448, camostat mesylate, sodium glycocholate, an amino acid, a modified amino acid, a peptide, a modified peptide, a polypeptide protease inhibitor, a complexing agent, a mucoadhesive polymer, a polymer-inhibitor conjugate, or a mixture thereof.

30 48. The pharmaceutical system of claim 46, wherein the enzyme inhibiting agent is selected from the group consisting of P-aminobenzamidine, FK-448, camostat mesylate, sodium glycocholate, aminoboronic acid derivatives, n-acetylcysteine, bacitracin, phosphinic acid dipeptide derivatives, pepstatin, antipain, leupeptin, chymostatin, elastatin, bestatin, phosphoramidon, puromycin, cytochalasin potatocarboxy

1 peptidase inhibitor, amastatin, protinin, Bowman-Birk inhibitor, soybean trypsin inhibitor,
chicken egg white trypsin inhibitor, chicken ovoinhibitor, human pancreatic trypsin
inhibitor, EDTA, EGTA, 1,10-phenanthroline, hydroxychinoline, polyacrylate derivatives,
chitosan, celluloses, chitosan-EDTA, chitosan-EDTA-antipain, polyacrylic acid-
bacitracin, carboxymethyl cellulose-pepstatin, polyacrylic acid-Bowman-Birk inhibitor,
and mixtures thereof.

49. The pharmaceutical system of claim 1, wherein the composition further
comprises an aqueous medium comprising water, an aqueous palatable diluent or an
aqueous beverage.

10 50. The pharmaceutical system of claim 49, wherein the therapeutic agent is
provided to the system in the aqueous medium.

51. The pharmaceutical system of claim 49, wherein the aqueous medium
further comprises an amount of an enzyme inhibiting agent sufficient to at least partially
inhibit enzymatic degradation of the hydrophilic therapeutic agent, the enzyme inhibiting
15 agent being solubilized, suspended, or partially solubilized and partially suspended, in the
aqueous medium.

52. The pharmaceutical system of claim 1, wherein the composition further
comprises a pharmaceutically acceptable acid.

53. The pharmaceutical system of claim 52, wherein the acid is selected from
20 the group consisting of hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid,
carbonic acid, nitric acid, boric acid, phosphoric acid, acetic acid, acrylic acid, adipic acid,
alginic acid, alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid,
butyric acid, carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic
acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic
25 acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid,
salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid,
toluenesulfonic acid, uric acid, and mixtures thereof.

54. The pharmaceutical system of claim 1, wherein the composition further
comprises a pharmaceutically acceptable base.

30 55. The pharmaceutical system of claim 54, wherein the base is an amino acid,
an amino acid ester, ammonium hydroxide, potassium hydroxide, sodium hydroxide,
sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate, magnesium

1 hydroxide, magnesium aluminum silicate, synthetic aluminum silicate, synthetic
hydrocalcite, magnesium aluminum hydroxide, diisopropylethylamine, ethanolamine,
ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, or a salt of a
pharmaceutically acceptable cation and acetic acid, acrylic acid, adipic acid, alginic acid,
5 alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid, butyric acid,
carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic acid,
hydroquinolsulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic acid,
oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid,
salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid,
10 toluenesulfonic acid, and uric acid, or a mixture thereof.

56. The pharmaceutical system of claim 1, wherein the at least two surfactants
are present in amounts such that the composition forms an aqueous dispersion having an
average particle size of less than about 200 nm upon mixing with an aqueous diluent.

57. The pharmaceutical system of claim 56, wherein the average particle size is
15 less than about 100 nm.

58. The pharmaceutical system of claim 56, wherein the average particle size is
less than about 50 nm.

59. The pharmaceutical system of claim 1, wherein the at least two surfactants
are present in amounts such that the composition forms a substantially optically clear
20 aqueous dispersion upon mixing with an aqueous diluent.

60. The pharmaceutical system of claim 1, wherein the system is substantially
free of polyethylene glycol diesters.

61. The pharmaceutical system of claim 1, wherein the system is substantially
free of cholesterol.

25 62. The pharmaceutical system of claim 1, wherein the dosage form is
substantially free of water.

63. The pharmaceutical system of claim 1 in the form of a preconcentrate in a
liquid, semi-solid, or solid form, or as an aqueous or organic diluted preconcentrate.

30 64. The pharmaceutical system of claim 1, wherein the dosage form of the
composition is processed by balling, lyophilization, encapsulation, extruding,
compression, melting, molding, spraying, spray congealing, coating, comminution,

1 mixing, cryopelletization, spheronization, homogenization, sonication, granulation, or a combination thereof.

65. The pharmaceutical system of claim 1, wherein the dosage form of the composition of is as a pill, capsule, caplet, tablet, granule, pellet, bead or powder.

5 66. The pharmaceutical system of claim 1, wherein the dosage form of the composition is a starch capsule, a cellulosic capsule, a hard gelatin capsule or a soft gelatin capsule.

10 67. The pharmaceutical system of claim 1, wherein the dosage form is formulated for immediate release, controlled release, extended release, delayed release, targeted release, or targeted delayed release.

68. The pharmaceutical system of claim 65, which further comprises at least one enteric coating, seal coating, extended release coating, or targeted delayed release coating.

15 69. The pharmaceutical system of claim 68, wherein the coating is formed of a material selected from the group consisting of shellac, acrylic polymers, cellulosic derivatives, polyvinyl acetate phthalate, and mixtures thereof.

20 70. The pharmaceutical system of claim 68, wherein the coating is formed of a material selected from the group consisting of Eudragit E, Eudragit L, Eudragit S, Eudragit RL, Eudragit RS, Eudragit NE, Eudragit L.RTM, Eudragit L300.RTM, Eudragit S.RTM, Eudragit L100-55RTM, cellulose acetate phthalate, Aquateric, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose succinate, polyvinylacetate phthalate, Cotteric, and mixtures thereof.

25 71. The pharmaceutical system of claim 68, wherein the coating is formed of a material selected from the group consisting of Eudragit L.RTM, Eudragit L300.RTM, Eudragit S.RTM, Eudragit L100-55RTM, cellulose acetate phthalate, Aquateric, ethyl cellulose, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose succinate, polyvinylacetate phthalate, Cotteric, and mixtures thereof.

30 72. The pharmaceutical system of claim 1, wherein the dosage form of the composition is a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup or elixir.

73. The pharmaceutical system of claim 1, wherein the dosage form is a multiparticulate carrier coated onto a substrate with the composition.

1 74. The pharmaceutical system of claim 73, wherein the substrate is a particle, a granule, a pellet or a bead, and is formed of the therapeutic agent, a pharmaceutically acceptable material, or a mixture thereof.

5 75. The pharmaceutical system of claim 73, wherein the multiparticulate carrier is coated with at least one enteric coating, seal coating, extended release coating, or targeted delayed release coating.

10 76. The pharmaceutical system of claim 73, wherein the dosage form is further processed by encapsulation, compression, extrusion, molding, spheronization or cryopelletization.

15 77. The pharmaceutical system of claim 73, wherein the dosage form is further processed to form a starch capsule, a cellulosic capsule, a hard gelatin capsule, or a soft gelatin capsule.

15 78. The pharmaceutical system of claim 77, wherein the capsule is coated with at least one enteric coating, seal coating, extended release coating, or targeted delayed release coating.

18 79. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent is present in the dosage form of the composition.

20 80. The pharmaceutical system of claim 79, wherein the hydrophilic therapeutic agent is solubilized in the composition, suspended in the composition, or partially solubilized and partially suspended in the composition.

25 81. The pharmaceutical system of claim 1, wherein the hydrophilic therapeutic agent is present in a dosage form separate from the dosage form of the composition.

25 82. The pharmaceutical system of claim 1, wherein the dosage form of the composition is formulated for oral, mucosal, nasal, pulmonary, vaginal, transmembrane, buccal or rectal administration.

28 83. The pharmaceutical system of claim 81, wherein the dosage form of the hydrophilic therapeutic agent is formulated for oral, mucosal, nasal, pulmonary, vaginal, transmembrane, buccal or rectal administration.

30 84. A pharmaceutical system for enhanced absorption of a hydrophilic therapeutic agent, the system comprising:

- 1 (a) a dosage form of an absorption enhancing composition, the
composition comprising at least one hydrophilic surfactant and at least one
hydrophobic surfactant; and
- 5 (b) a hydrophilic therapeutic agent,
- the pharmaceutical system being substantially free of triglycerides.
85. The pharmaceutical system of claim 84, wherein the hydrophilic surfactant
comprises at least one ionized ionizable surfactant;
86. The pharmaceutical system of claim 85, wherein the ionized ionizable
surfactant is the ionized form of a surfactant selected from the group consisting of bile
10 acids and salts, analogues, and derivatives thereof; lecithins, lysolecithin, phospholipids,
lysophospholipids and derivatives thereof; carnitine fatty acid ester salts; salts of
alkylsulfates; salts of fatty acids; sodium docusate; acyl lactylates; mono-, diacetylated
tartaric acid esters of mono-, diglycerides; succinylated monoglycerides; citric acid esters
of mono-, diglycerides; and mixtures thereof.
- 15 87. The pharmaceutical system of claim 85, wherein the ionized ionizable
surfactant is the ionized form of a surfactant selected from the group consisting of lecithin,
lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol,
phosphatidic acid, phosphatidylserine, lysophosphatidylcholine,
20 lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid,
lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine,
lactyl esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated
monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid
esters of mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate,
25 taurodeoxycholate, chenodeoxycholate, glycodeoxycholate, glycochenodeoxycholate,
taurochenodeoxycholate, ursodeoxycholate, lithocholate, tauroursodeoxycholate,
glycoursoodeoxycholate, cholylsarcosine, N-methyl taurocholate, caproate, caprylate,
caprate, laurate, myristate, palmitate, oleate, ricinoleate, linoleate, linolenate, stearate,
lauryl sulfate, tetraacetyl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl
30 carnitine, and salts and mixtures thereof.
88. The pharmaceutical system of claim 85, wherein the ionized ionizable
surfactant is the ionized form of a surfactant selected from the group consisting of lecithin,
lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol,

1 lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholate, taurocholate, glycocholate, deoxycholate, chenodeoxycholate, lithocholate,
5 ursodeoxycholate, taurodeoxycholate, glycodeoxycholate, cholylsarcosine, caproate, caprylate, caprate, laurate, oleate, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and salts and mixtures thereof.

89. The pharmaceutical system of claim 85, wherein the ionized ionizable surfactant is the ionized form of a surfactant selected from the group consisting of lecithin, 10 lactylic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, chenodeoxycholate, lithocholate, ursodeoxycholate, taurocholate, caprylate, caprate, oleate, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and salts and mixtures thereof.

15 90. The pharmaceutical system of claim 84, wherein the hydrophilic surfactant comprises at least one non-ionic hydrophilic surfactant having an HLB value greater than or equal to about 10.

91. The pharmaceutical system of claim 90, wherein the non-ionic surfactant is selected from the group consisting of alkylglucosides; alkylmaltosides; 20 alkylthioglucosides; lauryl macroglycerides; polyoxyethylene alkyl ethers; polyoxyethylene alkylphenols; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene sterols, derivatives, and analogues thereof; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, 25 hydrogenated vegetable oils, and sterols; sugar esters, sugar ethers; sucroglycerides; and mixtures thereof.

92. The pharmaceutical system of claim 90, wherein the non-ionic hydrophilic 30 surfactant is selected from the group consisting of polyoxyethylene alkylethers; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block

1 copolymers; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

5 93. The pharmaceutical system of claim 92, wherein the glyceride is a monoglyceride, diglyceride, triglyceride, or a mixture thereof.

94. The pharmaceutical system of claim 92, wherein the reaction mixture comprises the transesterification products of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and
10 sterols.

95. The pharmaceutical system of claim 92, wherein the polyol is glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, or a mixture thereof.

96. The pharmaceutical system of claim 90, wherein the hydrophilic surfactant
15 is PEG-10 laurate, PEG-12 laurate, PEG-20 laurate, PEG-32 laurate, PEG-32 dilaurate, PEG-12 oleate, PEG-15 oleate, PEG-20 oleate, PEG-20 dioleate, PEG-32 oleate, PEG-200
oleate, PEG-400 oleate, PEG-15 stearate, PEG-32 distearate, PEG-40 stearate, PEG-100
stearate, PEG-20 dilaurate, PEG-25 glyceryl trioleate, PEG-32 dioleate, PEG-20 glyceryl
20 laurate, PEG-30 glyceryl laurate, PEG-20 glyceryl stearate, PEG-20 glyceryl oleate, PEG-
30 glyceryl oleate, PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-40 palm kernel
oil, PEG-50 hydrogenated castor oil, PEG-40 castor oil, PEG-35 castor oil, PEG-60 castor
oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-60 corn oil,
PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polyglyceryl-10
25 laurate, PEG-30 cholesterol, PEG-25 phytosterol, PEG-30 soya sterol, PEG-20 trioleate,
PEG-40 sorbitan oleate, PEG-80 sorbitan laurate, polysorbate 20, polysorbate 80, POE-9
lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, POE-20 oleyl ether, POE-20 stearyl
ether, tocopheryl PEG-100 succinate, PEG-24 cholesterol, polyglyceryl-10 oleate, Tween
40, Tween 60, sucrose monostearate, sucrose monolaurate, sucrose monopalmitate, PEG
30 10-100 nonyl phenol series, PEG 15-100 octyl phenol series, a poloxamer, or a mixture
thereof.

97. The pharmaceutical system of claim 90, wherein the hydrophilic surfactant
is PEG-20 laurate, PEG-20 oleate, PEG-35 castor oil, PEG-40 palm kernel oil, PEG-40

1 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, polyglyceryl-10 laurate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, PEG-30 cholesterol, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, PEG-24 cholesterol, sucrose monostearate, sucrose monolaurate, a
5 poloxamer, or a mixture thereof.

98. The pharmaceutical system of claim 90, wherein the hydrophilic surfactant is PEG-35 castor oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polysorbate 20, polysorbate 80, tocopheryl PEG-1000 succinate, PEG-24 cholesterol, a
10 poloxamer, or a mixture thereof.

99. The pharmaceutical system of claim 84, wherein the composition comprises at least two hydrophilic surfactants.

100. The pharmaceutical system of claim 84, wherein the hydrophobic surfactant comprises an un-ionized ionizable surfactant.

101. The pharmaceutical system of claim 100, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of bile acids and analogues and derivatives thereof; lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; carnitine fatty acid esters; alkylsulfates; fatty acids; acyl lactylates; mono-,diacetylated tartaric acid esters of mono-,diglycerides; 20 succinylated monoglycerides; citric acid esters of mono-,diglycerides; and mixtures thereof.

102. The pharmaceutical system of claim 100, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, 25 phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid, lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine, lactyl esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid 30 esters of mono/diglycerides, cholic acid, taurocholic acid, glycocholic acid, deoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid, glycdeoxycholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, ursodeoxycholic acid,

1 lithocholic acid, taouroursodeoxycholic acid, glycoursoodeoxycholic acid, cholylsarcosine,
N-methyl taurocholic acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic
acid, palmitic acid, oleic acid, ricinoleic acid, linoleic acid, linolenic acid, stearic acid,
5 lauryl sulfate, tetraacetyl sulfate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine,
and mixtures thereof.

103. The pharmaceutical system of claim 100, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysophosphatidylcholine, PEG-phosphatidylethanolamine, lactic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, cholic acid, taurocholic acid, glycocholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid, taurodeoxycholic acid, glycodeoxycholic acid, cholylsarcosine, caproic acid, caprylic acid, capric acid, lauric 15 acid, oleic acid, lauryl sulfate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and mixtures thereof.

104. The pharmaceutical system of claim 100, wherein the un-ionized ionizable surfactant is the un-ionized form of a surfactant selected from the group consisting of lecithin, lactic esters of fatty acids, stearoyl-2-lactylate, stearoyl lactylate, succinylated 20 monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid, taurocholic acid, caprylic acid, capric acid, oleic acid, lauryl sulfate, docusate, lauroyl carnitine, palmitoyl carnitine, myristoyl carnitine, and mixtures thereof.

105. The pharmaceutical system of claim 84 wherein the hydrophobic surfactant 25 comprises at least one compound having an HLB value less than about 10.

106. The pharmaceutical system of claim 105, wherein the hydrophobic surfactant is selected from the group consisting of alcohols; polyoxyethylene alkylethers; fatty acids; bile acids; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower 30 alcohol fatty acids esters; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; lactic acid derivatives of mono/diglycerides; propylene glycol diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-

1 polyoxypropylene block copolymers; transesterified vegetable oils; sterols; sterol derivatives; sugar esters; sugar ethers; sucroglycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated
5 vegetable oils, and sterols; and mixtures thereof.

107. The pharmaceutical system of claim 105, wherein the hydrophobic surfactant is selected from the group consisting of fatty acids; bile acids; lower alcohol fatty acid esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; glycerol fatty acid esters; acetylated glycerol fatty
10 acid esters; lactic acid derivatives of mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures
15 thereof.

108. The pharmaceutical system of claim 105, wherein the hydrophobic surfactant is selected from the group consisting of bile acids; lower alcohol fatty acids esters; polypropylene glycol fatty acid esters; propylene glycol fatty acid esters; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lactic acid derivatives of mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene vegetable oils; and mixtures thereof.

109. The pharmaceutical system of claim 105, wherein the hydrophobic surfactant is a glycerol fatty acid ester, an acetylated glycerol fatty acid ester, or a mixture thereof.

110. The pharmaceutical system of claim 109, wherein the glycerol fatty acid ester is a monoglyceride, diglyceride, or a mixture thereof.

111. The pharmaceutical system of claim 110, wherein the fatty acid of the glycerol fatty acid ester is a C₆ to C₂₂ fatty acid or a mixture thereof.

112. The pharmaceutical system of claim 105, wherein the hydrophobic
30 surfactant is a reaction mixture of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

1 113. The pharmaceutical system of claim 112, wherein the reaction mixture is a
transesterification product of a polyol and at least one member of the group consisting of
fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

5 114. The pharmaceutical system of claim 112, wherein the polyol is
polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, or a mixture
thereof.

10 115. The pharmaceutical system of claim 105, wherein the hydrophobic
surfactant is selected from the group consisting of myristic acid; oleic acid; lauric acid;
stearic acid; palmitic acid; PEG 1-4 stearate; PEG 2-4 oleate; PEG-4 dilaurate; PEG-4
dioleate; PEG-4 distearate; PEG-6 dioleate; PEG-6 distearate; PEG-8 dioleate; PEG 3-16
castor oil; PEG 5-10 hydrogenated castor oil; PEG 6-20 corn oil; PEG 6-20 almond oil;
PEG-6 olive oil; PEG-6 peanut oil; PEG-6 palm kernel oil; PEG-6 hydrogenated palm
kernel oil; PEG-4 capric/caprylic triglyceride, mono, di, tri, tetra esters of vegetable oil
and sorbitol; pentaerythrityl di, tetra stearate, isostearate, oleate, caprylate, or caprate;
15 polyglyceryl 2-4 oleate, stearate, or isostearate; polyglyceryl 4-10 pentaoleate;
polyglyceryl-3 dioleate; polyglyceryl-6 dioleate; polyglyceryl-10 trioleate; polyglyceryl-3
distearate; propylene glycol mono- or diesters of a C₆ to C₂₂ fatty acid; monoglycerides of
a C₆ to C₂₂ fatty acid; acetylated monoglycerides of C₆ to C₂₂ fatty acid; diglycerides of C₆
to C₂₂ fatty acids; lactic acid derivatives of monoglycerides; lactic acid derivatives of
20 diglycerides; cholesterol; phytosterol; PEG 5-20 soya sterol; PEG-6 sorbitan tetra,
hexastearate; PEG-6 sorbitan tetraoleate; sorbitan monolaurate; sorbitan monopalmitate;
sorbitan mono, trioleate; sorbitan mono, tristearate; sorbitan monoisostearate; sorbitan
sesquioleate; sorbitan sesquistearate; PEG 2-5 oleyl ether; POE 2-4 lauryl ether; PEG-2
cetyl ether; PEG-2 stearyl ether; sucrose distearate; sucrose dipalmitate; ethyl oleate;
25 isopropyl myristate; isopropyl palmitate; ethyl linoleate; isopropyl linoleate; poloxamers;
cholic acid; ursodeoxycholic acid; glycocholic acid; taurocholic acid; lithocholic acid;
deoxycholic acid; chenodeoxycholic acid; and mixtures thereof.

30 116. The pharmaceutical system of claim 105, wherein the hydrophobic
surfactant is selected from the group consisting of oleic acid; lauric acid; glyceryl
monocaprate; glyceryl monocaprylate; glyceryl monolaurate; glyceryl monooleate;
glyceryl dicaprate; glyceryl dicaprylate; glyceryl dilaurate; glyceryl dioleate; acetylated
monoglycerides; propylene glycol oleate; propylene glycol laurate; polyglyceryl-3 oleate;

- 1 polyglyceryl-6 dioleate; PEG-6 corn oil; PEG-20 corn oil; PEG-20 almond oil; sorbitan monooleate; sorbitan monolaurate; POE-4 lauryl ether; POE-3 oleyl ether; ethyl oleate; poloxamers; cholic acid; ursodeoxycholic acid; glycocholic acid; taurocholic acid; lithocholic acid; deoxycholic acid; chenodeoxycholic acid; and mixtures thereof.
- 5 117. The pharmaceutical system of claim 84, wherein the hydrophobic and hydrophilic surfactants are selected from the hydrophobic and hydrophilic members, respectively, of the group consisting of sodium lauryl sulfate, oleic acid, linoleic acid, monoolein, lecithin, lysolecithin, deoxycholate, taurodeoxycholate, glycochenodeoxycholate, polyoxyethylene X-lauryl ether, where X is from 9 to 20,
- 10 sodium tauro-24,25-dihydrofusidate, polyoxyethylene ether, polyoxyethylene sorbitan esters, p-t-octylphenoxy polyoxyethylene, N-lauryl- β -D-maltopyranoside, 1-dodecylazacycloheptane-2-azone, and phospholipids, and are each present in an amount of greater than 10% by weight, based on the total weight of the pharmaceutical system.
- 15 118. The pharmaceutical system of claim 84, wherein the hydrophilic therapeutic agent is a drug, a vitamin, a nutritional supplement, a cosmeceutical, a diagnostic agent, or a mixture thereof.
119. The pharmaceutical system of claim 84, wherein the hydrophilic therapeutic agent has an apparent water solubility of at least about 1 mg/mL.
- 20 120. The pharmaceutical system of claim 84, wherein the hydrophilic therapeutic agent is a hydrophilic drug, a cytokine, a peptidomimetic, a peptide, a protein, a toxoid, a serum, an antibody, a vaccine, a nucleoside, a nucleotide, a portion of genetic material, a nucleic acid, or a mixture thereof.
- 25 121. The pharmaceutical system of claim 84, wherein the hydrophilic therapeutic agent is selected from the hydrophilic members of the group consisting of analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, anti-asthma agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents, immunosuppressants, anti-protozoal agents, anti-thyroid agents, anti-tussives, anxiolytic, sedatives, hypnotics, neuroleptics, β -Blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonian agents, gastro-intestinal agents, histamine H₂-receptor antagonists, keratolytics, lipid regulating agents, muscle relaxants, anti-anginal agents,

- 1 nutritional agents, analgesics, sex hormones, stimulants, cytokines, peptidomimetics,
peptides, proteins, toxoids, sera, antibodies, vaccines, nucleosides, nucleotides, genetic
material, nucleic acids, and mixtures thereof.
- 5 122. The pharmaceutical system of claim 84, wherein the hydrophilic
therapeutic agent is selected from the group consisting of acarbose; acyclovir; acetyl
cysteine; acetylcholine chloride; alatrofloxacin; alendronate; alglycerase; amantadine
hydrochloride; ambenonium; amifostine; amiloride hydrochloride; aminocaproic acid;
amphotericin B; antihemophilic factor (human); antihemophilic factor (porcine);
antihemophilic factor (recombinant); aprotinin; asparaginase; atenolol; atracurium
10 besylate; atropine; azithromycin; aztreonam; BCG vaccine; bacitracin; becalermin;
belladona; bepridil hydrochloride; bleomycin sulfate; calcitonin human; calcitonin salmon;
carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium;
cefepime hydrochloride; cefixime; cefonidic sodium; cefoperazone; cefotetan disodium;
cefotoxime; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalixin;
15 cephepirin sodium; cholera vaccine; chronic gonadotropin; cidofovir; cisplatin;
cladribine; clidinium bromide; clindamycin and clindamycin derivatives; ciprofloxacin;
clondronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromalyin
sodium; cytarabine; daltaperin sodium; danaproid; deforoxamine; denileukin diftitox;
desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine;
20 dirithromycin; dopamine hydrochloride; domase alpha; doxacurium chloride; doxorubicin;
editronate disodium; elanaprilat; enkephalin; enoxacin; enoxaprin sodium; ephedrine;
epinephrine; epoetin alpha; erythromycin; esmol hydrochloride; factor IX; famiciclovir;
fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating
25 factor; granulocyte-macrophage stimulating factor; growth hormones- recombinant
human; growth hormone- bovine; gentamycin; glucagon; glycopyrrolate; gonadotropin
releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; grepafloxacin;
hemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus
vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine;
interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin procine; insulin NPH;
30 insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta;
ipratropium bromide; isofosfamide; japanese encephalitis virus vaccine; lamivudine;
leucovorin calcium; leuprolide acetate; levofloxacin; lincomycin and lincomycin

1 derivatives; lobucavir; lomefloxacin; loracarbef; mannitol; measles virus vaccine;
meningococcal vaccine; menotropins; mephenzolate bromide; mesalmine; methanamine;
methotrexate; methscopolamine; metformin hydrochloride; metroprolol; mezocilllin
sodium; mivacurium chloride; mumps viral vaccine; nedocromil sodium; neostigmine
5 bromide; neostigmine methyl sulfate; neutontin; norfloxacin; octreotide acetate; ofloxacin;
olpadronate; oxytocin; pamidronate disodium; pancuronium bromide; paroxetine;
pefloxacin; pentamidine isethionate; pentostatin; pentoxifylline; periclovir;
pentagastrin; phentolamine mesylate; phenylalanine; physostigmine salicylate; plague
10 vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine
polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymixin B
sulfate; pralidoxine chloride; pramlintide; pregabalin; propofenone; propenthaline
bromide; pyridostigmine bromide; rabies vaccine; residronate; ribavarin; rimantadine
hydrochloride; rotavirus vaccine; salmetrol xinafoate; sincalide; small pox vaccine;
15 solatol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin;
suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiopeta; ticarcillin;
tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; trandolapril;
trimetrexate gluconate; trospectinomycin; trovafloxacin; tubocurarine chloride; tumor
necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valaciclovir; valsartan;
varicella virus vaccine live; vasopressin and vasopressin derivatives; vecuronium bromide;
20 vinblastin; vincristine; vinorelbine; vitamin B12 ; warfarin sodium; yellow fever vaccine;
zalcitabine; zanamavir; zolandronate; and zidovudine.

123. The pharmaceutical system of claim 84, wherein the hydrophilic therapeutic agent is selected from the group consisting of acarbose; acyclovir; atracurium besylate; alendronate; alglucerase; amantadine hydrochloride; amphotericin B;
25 antihemophilic factor (human); antihemophilic factor (porcine); antihemophilic factor (recombinant; azithromycin; calcitonin human; calcitonin salmon; capecitabine; cefazolin sodium; cefoncid sodium; cefoperazone; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalexin; chrionic gonadotropin; cidofovir; cladribine ; clindamycin and clindamycin derivatives; corticotropin; cosyntropin; cromalyn sodium; cytarabine;
30 daltaperin sodium; danaproid; desmopressin; didanosine; dirithromycin; editronate disodium; enoxaprin sodium; epoetin alpha; factor IX; famiciclovir; fludarabine; foscarnet sodium; ganciclovir; granulocyte colony stimulating factor; granulocyte-macrophage

1 stimulating factor; growth hormones- recombinant human; growth hormone- Bovine; gentamycin; glucagon; gonadotropin releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; hemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate; 5 influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin procline; insulin NPH; insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide; isofosfamide; lamivudine; leucovorin calcium; leuprolide acetate; lincomycin and lincomycin derivatives; metformin hydrochloride; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neutontin; 10 octreotide acetate; olpadronate; pamidronate disodium; pancuronium bromide; pentamidine isethionate; pentagastrin; physostigmine salicylate; poliovirus vaccine live (OPV); pyridostigmine bromide; residronate; ribavarin; rimantadine hydrochloride; rotavirus vaccine; salmetrol xinafoate; somatostatin; spectinomycin; stavudine; streptokinase; ticarcillin; tiludronate; tissue type plasminogen activator; TNFR:Fc; TNK- 15 tPA; trimetrexate gluconate; trospectinomycin; tumor necrosis factor; typhoid vaccine live; urokinase; vancomycin; valaciclovir; vasopressin and vasopressin derivatives; vinblastin; vincristine; vinorelbine; warfarin sodium; zalcitabine; zanamavir; and zidovudine.

124. The pharmaceutical system of claim 84, wherein the hydrophilic 20 therapeutic agent is selected from the group consisting of acarbose; alendronate; amantadine hydrochloride; azithromycin; calcitonin human; calcitonin salmon; ceftriaxone; cefuroxime axetil; chrionic gonadotropin; cromalyn sodium; daltaperin sodium; danaproid; desmopressin; didanosine; editronate disodium; enoxaprin sodium; epoetin alpha; factor IX; famciclovir; foscamet sodium; ganciclovir; granulocyte colony 25 stimulating factor; granulocyte-macrophage stimulating factor; growth hormones-recombinant human; growth hormone- Bovine; glucagon; gonadotropin releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin procline interferon alpha; interferon beta; leuprolide acetate; metformin hydrochloride; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neutontin; 30 octreotide acetate; olpadronate; pamidronate disodium; residronate; rimantadine hydrochloride; salmetrol xinafoate; somatostatin; stavudine; ticarcillin; tiludronate; tissue

1 type plasminogen activator; TNFR:Fc; TNK-tPA; tumor necrosis factor; typhoid vaccine
live; vancomycin; valaciclovir; vasopressin and vasopressin derivatives; zalcitabine;
zanamivir and zidovudine.

5 125. The pharmaceutical system of claim 84, wherein the composition further
comprises a solubilizer.

126. The pharmaceutical system of claim 125, wherein the solubilizer is selected
from the group consisting of alcohols, polyols, amides, esters, propylene glycol ethers and
mixtures thereof.

10 127. The pharmaceutical system of claim 84, wherein the composition further
comprises an antioxidant, a bufferant, an antifoaming agent, a detackifier, a preservative, a
chelating agent, a viscomodulator, a tonicifier, a flavorant, a colorant, an odorant, an
opacifier, a suspending agent, a binder, a filler, a plasticizer, a lubricant, or a mixture
thereof.

15 128. The pharmaceutical system of claim 84, wherein the composition further
comprises an amount of an enzyme inhibiting agent sufficient to at least partially inhibit
enzymatic degradation of the hydrophilic therapeutic agent.

20 129. The pharmaceutical system of claim 128, wherein the enzyme inhibiting
agent is P-aminobenzamidine, FK-448, camostat mesylate, sodium glycocholate, an amino
acid, a modified amino acid, a peptide, a modified peptide, a polypeptide protease
inhibitor, a complexing agent, a mucoadhesive polymer, a polymer-inhibitor conjugate, or
a mixture thereof.

25 130. The pharmaceutical system of claim 128, wherein the enzyme inhibiting
agent is selected from the group consisting of P-aminobenzamidine, FK-448, camostat
mesylate, sodium glycocholate, aminoboronic acid derivatives, n-acetylcysteine,
bacitracin, phosphinic acid dipeptide derivatives, pepstatin, antipain, leupeptin,
chymostatin, elastatin, bestatin, phosphoramindon, puromycin, cytochalasin potatocarboxy
peptidase inhibitor, amastatin, protinin, Bowman-Birk inhibitor, soybean trypsin inhibitor,
chicken egg white trypsin inhibitor, chicken ovoinhibitor, human pancreatic trypsin
inhibitor, EDTA, EGTA, 1,10-phenanthroline, hydroxychinoline, polyacrylate derivatives,
30 chitosan, celluloses, chitosan-EDTA, chitosan-EDTA-antipain, polyacrylic acid-
bacitracin, carboxymethyl cellulose-pepstatin, polyacrylic acid-Bowman-Birk inhibitor,
and mixtures thereof.

1 131. The pharmaceutical system of claim 84, wherein the composition further
comprises an aqueous medium comprising water, an aqueous palatable diluent or an
aqueous beverage.

5 132. The pharmaceutical system of claim 131, wherein the therapeutic agent is
provided to the system in the aqueous medium.

10 133. The pharmaceutical system of claim 131, wherein the aqueous medium
further comprises an amount of an enzyme inhibiting agent sufficient to at least partially
inhibit enzymatic degradation of the hydrophilic therapeutic agent, the enzyme inhibiting
agent being solubilized, suspended, or partially solubilized and partially suspended, in the
aqueous medium.

134. The pharmaceutical system of claim 84, wherein the composition further
comprises a pharmaceutically acceptable acid.

15 135. The pharmaceutical system of claim 134, wherein the acid is selected from
the group consisting of hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid,
carbonic acid, nitric acid, boric acid, phosphoric acid, acetic acid, acrylic acid, adipic acid,
alginic acid, alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid,
butyric acid, carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic
acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic
acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid,
20 salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid,
toluenesulfonic acid, uric acid, and mixtures thereof.

136. The pharmaceutical system of claim 84, wherein the composition further
comprises a pharmaceutically acceptable base.

25 137. The pharmaceutical system of claim 136, wherein the base is an amino
acid, an amino acid ester, ammonium hydroxide, potassium hydroxide, sodium hydroxide,
sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate, magnesium
hydroxide, magnesium aluminum silicate, synthetic aluminum silicate, synthetic
hydrotalcite, magnesium aluminum hydroxide, diisopropylethylamine, ethanolamine,
ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, or a salt of a
30 pharmaceutically acceptable cation and acetic acid, acrylic acid, adipic acid, alginic acid,
alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid, butyric acid,
carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic acid,

- 1 hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, and uric acid, or a mixture thereof.
- 5 138. The pharmaceutical system of claim 84, wherein the at least two surfactants are present in amounts such that the composition forms an aqueous dispersion having an average particle size of less than about 200 nm upon mixing with an aqueous diluent.
139. The pharmaceutical system of claim 138, wherein the average particle size is less than about 100 nm.
- 10 140. The pharmaceutical system of claim 138, wherein the average particle size is less than about 50 nm.
141. The pharmaceutical system of claim 84, wherein the at least two surfactants are present in amounts such that the composition forms an substantially optically clear aqueous dispersion upon mixing with an aqueous diluent.
- 15 142. The pharmaceutical system of claim 84, wherein the system is substantially free of polyethylene glycol diesters.
143. The pharmaceutical system of claim 84, wherein the system is substantially free of cholesterol.
- 20 144. The pharmaceutical system of claim 84, wherein the dosage form is substantially free of water.
145. The pharmaceutical system of claim 84 in the form of a preconcentrate in a liquid, semi-solid, or solid form, or as an aqueous or organic diluted preconcentrate.
- 25 146. The pharmaceutical system of claim 84, wherein the dosage form of the composition is processed by balling, lyophilization, encapsulation, extruding, compression, melting, molding, spraying, spray congealing, coating, comminution, mixing, cryopelletization, spheronization, homogenization, sonication, granulation, or a combination thereof.
147. The pharmaceutical system of claim 84, wherein the dosage form of the composition is as a pill, capsule, caplet, tablet, granule, pellet, bead or powder.
- 30 148. The pharmaceutical system of claim 84, wherein the dosage form of the composition is a starch capsule, a cellulosic capsule, a hard gelatin capsule or a soft gelatin capsule.

1 149. The pharmaceutical system of claim 84, wherein the dosage form is
formulated for immediate release, controlled release, extended release, delayed release,
targeted release, or targeted delayed release.

5 150. The pharmaceutical system of claim 147, which further comprises at least
one enteric coating, seal coating, extended release coating, or targeted delayed release
coating.

10 151. The pharmaceutical system of claim 150, wherein the coating is formed of
a material selected from the group consisting of shellac, acrylic polymers, cellulosic
derivatives, polyvinyl acetate phthalate, and mixtures thereof.

15 152. The pharmaceutical system of claim 150, wherein the coating is formed of
a material selected from the group consisting of Eudragit E, Eudragit L, Eudragit S,
Eudragit RL, Eudragit RS, Eudragit NE, Eudragit L.RTM, Eudragit L300.RTM, Eudragit
S.RTM, Eudragit L100-55RTM, cellulose acetate phthalate, Aquateric, cellulose acetate
trimellitate, ethyl cellulose, hydroxypropyl methyl cellulose phthalate, hydroxypropyl
methyl cellulose succinate, polyvinylacetate phthalate, Cotteric, and mixtures thereof.

20 153. The pharmaceutical system of claim 150, wherein the coating is formed of
a material selected from the group consisting of Eudragit L.RTM, Eudragit L300.RTM,
Eudragit S.RTM, Eudragit L100-55RTM, cellulose acetate phthalate, Aquateric, ethyl
cellulose, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose
succinate, polyvinylacetate phthalate, Cotteric, and mixtures thereof.

25 154. The pharmaceutical system of claim 84, wherein the dosage form of the
composition is a solution, suspension, emulsion, cream, ointment, lotion, suppository,
spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup or elixir.

155. The pharmaceutical system of claim 84, wherein the dosage form is a
multiparticulate carrier coated onto a substrate with the composition.

30 156. The pharmaceutical system of claim 155, wherein the substrate is a particle,
a granule, a pellet or a bead, and is formed of the therapeutic agent, a pharmaceutically
acceptable material, or a mixture thereof.

157. The pharmaceutical system of claim 155, wherein the multiparticulate
carrier is coated with at least one enteric coating, seal coating, extended release coating, or
targeted delayed release coating.

- 1 158. The pharmaceutical system of claim 155, wherein the dosage form is
further processed by encapsulation, compression, extrusion, molding, spheronization or
cryopelletization.
- 5 159. The pharmaceutical system of claim 155, wherein the dosage form is
further processed to form a starch capsule, a cellulosic capsule, a hard gelatin capsule, or a
soft gelatin capsule.
- 10 160. The pharmaceutical system of claim 159, wherein the capsule is coated
with at least one enteric coating, seal coating, extended release coating, or targeted
delayed release coating.
- 15 161. The pharmaceutical system of claim 84, wherein the hydrophilic
therapeutic agent is present in the dosage form of the composition.
- 20 162. The pharmaceutical system of claim 161, wherein the hydrophilic
therapeutic agent is solubilized in the composition, suspended in the composition, or
partially solubilized and partially suspended in the composition.
- 25 163. The pharmaceutical system of claim 84, wherein the hydrophilic
therapeutic agent is present in a dosage form separate from the dosage form of the
composition.
- 30 164. The pharmaceutical system of claim 84, wherein the dosage form of the
composition is formulated for oral, mucosal, pulmonary, nasal, vaginal, transmembrane,
buccal or rectal administration.
165. The pharmaceutical system of claim 163, wherein the dosage form of the
hydrophilic therapeutic agent is formulated for oral, mucosal, pulmonary, nasal, vaginal,
transmembrane, buccal or rectal administration.
166. An absorption enhancing composition for co-administration to a patient
with a hydrophilic therapeutic agent, the composition comprising an effective amount of
an absorption enhancer comprising at least two surfactants, at least one of which is
hydrophilic, the absorption enhancing composition being substantially triglyceride free.
167. The composition of claim 166, wherein the effective amount is an amount
sufficient to increase the rate, the extent, or both the rate and extent, of bioabsorption of a
hydrophilic therapeutic agent, when the composition and the hydrophilic therapeutic agent
are administered to a patient.

1 168. The composition of claim 166, wherein the effective amount is an amount sufficient to improve the consistency of the rate, the extent, or both the rate and extent, of bioabsorption of a hydrophilic therapeutic agent, when the composition and the hydrophilic therapeutic agent are administered to a patient.

5 169. The composition of claim 166, which further comprises a hydrophilic therapeutic agent.

10 170. A method of controlling the rate, the extent, or both the rate and extent, of bioabsorption of a hydrophilic therapeutic agent administered to a patient, the method comprising:

15 (a) providing a dosage form of an absorption enhancing composition, the composition comprising at least two surfactants, at least one of which is hydrophilic, and being substantially free of triglycerides;

16 (b) providing a hydrophilic therapeutic agent; and

17 (c) administering the dosage form of the absorption enhancing composition and the hydrophilic therapeutic agent to the patient.

20 171. The method of claim 170, wherein the hydrophilic therapeutic agent is provided in the dosage form of the absorption enhancing composition.

25 172. The method of claim 171, wherein the hydrophilic therapeutic agent is solubilized, suspended, or partially solubilized and partially suspended, in the dosage form of the absorption enhancing composition.

30 173. The method of claim 170, wherein the hydrophilic therapeutic agent is provided in a dosage form separate from the dosage form of the absorption enhancing composition.

25 174. The method of claim 173, wherein the step of administering comprises administering the dosage form of the absorption enhancing composition and co-administering the dosage form of the hydrophilic therapeutic agent.

35 175. The method of claim 170, wherein the dosage form of the absorption enhancing composition is formulated for oral, mucosal, pulmonary, nasal, vaginal, transmembrane, buccal or rectal administration.

30 176. The method of claim 173, wherein the dosage form of the hydrophilic therapeutic agent is formulated for oral, mucosal, pulmonary, nasal, vaginal, transmembrane, buccal or rectal administration.

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- 1 177. The method of claim 170, wherein the patient is a mammal.
178. The method of claim 170, wherein the patient is a human.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/18807

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 9/00, 9/14, 9/16, 9/20, 9/22, 9/28, 9/48
 US CL : 424/451, 456, 457, 464, 489

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/451, 456, 457, 464, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| X | US 5,858,398 A (CHO) 12 January 1999, abstract, columns 11-18, examples and claims. | 1-178 |

 Further documents are listed in the continuation of Box C. See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/18807

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST

search terms: hydrophilic, hydrophobic, surfactants, phospholipids, sterols, cholesterol, pills, tablets, capsules, powders, beads.



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| <p>(54) Title: COMPOSITIONS COMPRISING MICROPARTICLES OF WATER-INSOLUBLE SUBSTANCES AND METHOD FOR PREPARING SAME</p> <p>(57) Abstract</p> <p>Submicron size particles of pharmaceutical or other water-insoluble or poorly water-insoluble substances are prepared using a combination of one or more surface modifiers/surfactants such as poloxamers, poloxamines, polyoxyethylene sorbitan fatty acid esters and the like together with natural or synthetic phospholipids. Particles so produced have a volume weighted mean particle size at least one-half smaller than obtainable using a phospholipid alone. Compositions so prepared are resistant to particle size growth on storage.</p> | | | |

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**COMPOSITIONS COMPRISING MICROPARTICLES OF WATER-INSOLUBLE SUBSTANCES AND
METHOD FOR PREPARING SAME**

This invention relates to compositions and procedures that yield
5 sub-micron and micron stable particles of water-insoluble or poorly
soluble drugs or other industrially useful insoluble compounds. The
compositions of this invention include combinations of natural or
synthetic phospholipids, and one or more non-ionic, anionic or
cationic surfactants coated or adhered onto the surfaces of the water
10 insoluble-compound particles. The combination of phospholipids and
surfactants allows the formation and stabilization of the sub-micron
and micron size compound particles via hydrophilic, lipophilic and
electrostatic interactions and therefore prevent these particles from
15 aggregation or flocculation.

15

BACKGROUND OF THE INVENTION

There is a critical need in the pharmaceutical and other
biological based industries to formulate water-insoluble or poorly
20 soluble substances into formulations for oral, injectable, inhalation
and ophthalmic routes of delivery. Water insoluble compounds are
those having poor solubility in water, that is < 5 mg/ml at
physiological pH (6.5-7.4). Preferably their water solubility is <
25 1 mg/ml, more preferably < 0.1 mg/ml. It is desirable that the drug is
stable in water as a dispersion; otherwise a lyophilized or spray-dried
solid form may be desirable.

- As used herein, "micro" refers to a particle having diameter of from nanometers to micrometers. Microparticles, as used herein, refer to solid particles of irregular, non-spherical or spherical shapes.
- Formulations containing these microparticles provide some specific advantages over the unformulated non-micronized drug particles, which include improved oral bioavailability of drugs that are poorly absorbed from GI tract, development of injectable formulations that are currently available only in oral dosage form, less toxic injectable formulations that are currently prepared with organic solvents.
- sustained release of intramuscular injectable drugs that are currently administered through daily injection or constant infusion, and preparation of inhaled, ophthalmic formulation of drugs that otherwise could not be formulated for nasal or ocular use.
- Current technology for delivering insoluble drugs as described in US Patents 5,091,188; 5,091,187 and 4,725,442 focuses on (a) either coating small drug particles with natural or synthetic phospholipids or (b) dissolving the drug in a suitable lipophilic carrier and forming an emulsion stabilized with natural or semisynthetic phospholipids. One of the disadvantages of these formulations is that certain drug particles in suspension tend to grow over time because of the dissolution and reprecipitation phenomenon known as the "Oswald ripening".

DESCRIPTION OF THE INVENTION

25

The present invention focuses on preparing submicron size particles using a combination of surface modifier(s) with a phospholipid, and how the growth of particle size, and hence storage stability, is

controlled by adding a combination of surface modifier(s) with a phospholipid to the formulation.

- The use of a surface modifier or combination of surface
- 5 modifiers in addition to a phospholipid is characterized by its ability to result in volume weighted mean particle size values that are (i) at least 50% and preferably about 50-90% smaller than what can be achieved using phospholipid alone without the use of a surfactant with the same energy input, and (ii) provide compositions resistant to
- 10 particle size growth on storage. While resistance to particle size growth on storage was an objective of this invention we were surprised to observe a significant reduction in particle size with the addition of the surfactant. In order to achieve the advantages of the present invention it is necessary that the phospholipid and the
- 15 surfactant both be present at the time of particle size reduction or precipitation.

Although we do not wish to be bound by any particular theory, it appears that these surface modifiers generally, that is phospholipids and one or more surfactants, adsorb to the surfaces of drug particles.

20 and (a) convert lipophilic to hydrophilic surfaces with increased steric hindrance/stability, and (b) possibly modify zeta potential of surfaces with more charge repulsion stabilization. The concentrations of surface modifiers used in the process described here are normally

25 above their critical micelle concentrations (CMC) and hence facilitate the formation of sub-micron particles by stabilizing the particles.

Phospholipid and surface modifier(s) are adsorbed on to the surfaces of drug particles in sufficient quantity to retard drug particle growth, reduce drug average particle size from 5 to 100 μm to sub-micron and micron size particles by one or combination of methods known in the art, such as sonication, homogenization, milling, microfluidization, precipitation or recrystallization or precipitation from supercritical fluid, and maintain sub-micron and micron size particles on subsequent storage as suspension or solid dosage form.

The concentration of phospholipid or surface modifier in the suspension or solid dosage form can be present in the range of 0.1 to 50%, preferably 0.2 to 20%, and more preferably 0.5 to 10%.

The formulations prepared by this invention may be lyophilized into powders, which can be resuspended or filled into capsules or converted into granules or tablets with the addition of binders and other excipients known in the art of tablet making.

By industrially useful insoluble or poorly soluble compounds we include biologically useful compounds, imaging agents, pharmaceutically useful compounds and in particular drugs for human and veterinary medicine. Water insoluble compounds are those having a poor solubility in water, that is less than 5 mg/ml at a physiological pH of 6.5 to 7.4, although the water solubility may be less than 1 mg/ml and even less than 0.1 mg/ml.

Examples of some preferred water-insoluble drugs include immunosuppressive and immunoactive agents, antiviral and

- antifungal agents, antineoplastic agents, analgesic and anti-inflammatory agents, antibiotics, anti-epileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists,
- 5 neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and antarrhythmic, antihypertensive agents, antineoplastic agents, hormones, and nutrients. A detailed description of these and other suitable drugs may be found in *Remington's Pharmaceutical Sciences*.
- 10 18th edition, 1990, Mack Publishing Co. Philadelphia, PA.

The phospholipid may be any natural or synthetic phospholipid, for example phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol.

- 15 phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof. The phospholipid may be salted or desalted, hydrogenated or partially hydrogenated or natural semisynthetic or synthetic.

- 20 Examples of some suitable second surface modifiers include:
- (a) natural surfactants such as casein, gelatin, tragacanth, waxes, enteric resins, paraffin, acacia, gelatin, cholesterol esters and triglycerides, (b) nonionic surfactants such as polyoxyethylene fatty alcohol ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, poloxamers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline

cellulose, polyvinyl alcohol, polyvinylpyrrolidone, and synthetic phospholipids, (c) anionic surfactants such as potassium laurate, triethanolamine stearate, sodium lauryl sulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, negatively charged phospholipids (phosphatidyl glycerol, phosphatidyl inosite, phosphatidylserine, phosphatidic acid and their salts), and negatively charged glyceryl esters, sodium carboxymethylcellulose, and calcium carboxymethylcellulose, (d) cationic surfactants such as quaternary ammonium compounds, benzalkonium chloride,
10 cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride, (e) colloidal clays such as bentonite and veegum. A detailed description of these surfactants may be found in Remington's Pharmaceutical Sciences, and Theory and Practice of Industrial Pharmacy, Lachman et al. 1986

More specifically, examples of suitable second surface modifiers include one or combination of the following: poloxamers, such as Pluronic™ F68, F108 and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and
20 poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene-diamine available from BASF, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas. Tween 20, 40, 60 and 80,
25 which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, hydroxy propylmethylcellulose, dimyristoyl phosphatidylglycerol sodium salt

sodium dodecylsulfate, sodium deoxycholate, and cetyltrimethylammonium bromide.

- It is thought that some of the functions of the second surface modifier(s) as it relates to this invention are suppressing the process of Oswald Ripening and therefore maintaining the particle size, increasing the storage stability, minimizing sedimentation, and decreasing the particle growth during lyophilization and reconstitution; adhere or coat firmly onto the surfaces of
- 5 water-insoluble drug particles and therefore modify the interfaces between the particles and the liquid in the resulting formulations; increase the interface compatibility between water-insoluble drug particles and the liquid; and possibly to orient preferentially themselves with the hydrophilic portion sticking into the aqueous
 - 10 solution and the lipophilic portion strongly adsorbed at the water-insoluble drug particle surfaces
 - 15

- Considerable variations as to the identities and types of phospholipid and especially the surface active agent or agents should be expected depending upon the drug or active agent selected as the surface properties of these small particles are different. The most advantageous surface active agent for the insoluble drug will be apparent following empirical tests to identify the surfactant or surfactant system/combination resulting in the requisite particle size
- 20
 - 25 and particle size stability on storage over time.

Various procedures can be used to produce these stable sub-micron and micron size particles including mixing the insoluble

- substance with phospholipid and precipitating from a dissolved mixture of the substance, phospholipid and surfactant using other surfactants followed by sonication, milling, homogenization, microfluidization, and antisolvent and solvent precipitation. Mannitol
5 and other agents may be added to adjust the final formulation to isotonicity as well as a stabilizing aid during drying.

Unless otherwise specified, all parts and percentages reported herein are weight per unit volume (w/v), in which the volume in the
10 denominator represents the total volume of the system. Diameters of dimensions are given in millimeters ($\text{mm} = 10^{-3}$ meters), micrometers ($\mu\text{m} = 10^{-6}$ meters), nanometers ($\text{nm} = 10^{-9}$ meters) or Angstrom units (= 0.1 nm). Volumes are given in liters (L), milliliters ($\text{mL} = 10^{-3}\text{ L}$) and microliters ($\mu\text{L} = 10^{-6}\text{ L}$). Dilutions are by volume. All
15 temperatures are reported in degrees Celsius. The compositions of the invention can comprise, consist essentially of or consist of the materials set forth and the process or method can comprise, consist essentially of or consist of the steps set forth with such materials.

20 The following examples further explain and illustrate the invention:

Example 1

25 Microparticle-cyclosporine, of an immunosuppressive drug. was prepared as follows. The composition and concentration of excipients of the microparticle cyclosporine formulation are listed below:

| | |
|-------------------------|------------|
| Cyclosporine | 50 mg/ml |
| Egg Phosphatidylcholine | 100 mg/ml |
| Mannitol | 55 mg/ml |
| Tween 80 | 10 mg/ml |
| 5 Distilled Water | qs to 100% |
| Total Volume | 20 ml |

10 Cyclosporine with an average particle size from 5-100 μm , and
mannitol were purchased from Sigma, egg phosphatidylcholine was
produced by Pfanstiehl, Tween 80 was purchased from ICI.

The above components were placed in a 30 ml beaker and
pre-mixed with a hand-held biohomogenizer (Honeywell DR 4200
model GP) for 1-5 min. During homogenization, dilute NaOH was
15 added to the pre-mix to adjust the pH from 3.1 to 7 ± 0.5 . The
pre-mix was placed in a water jacketed vessel (50 ml capacity)
through which thermostated water at 4°C was circulated to control the
temperature of the formulation. The pre-mix was subjected to high
shear energy of a probe sonicator (Fisher, model 550 Sonic
20 Dismembrator) with a 0.5 inch diameter probe. Sonic pulses of 10
seconds at 10-seconds intervals at a power setting of 5 were utilized.
During sonication the temperature of the formulation was $18 \pm 2^\circ\text{C}$.
The pH during sonication was adjusted to 7 ± 0.5 with dilute NaOH.
Total sonication time employed to prepare the microparticle
25 cyclosporine was usually 10.5 hours or less. The microparticle-
cyclosporine formulation was placed in 20 ml vials and stored at 4
and 25°C for further stability studies.

Particle size distribution of the suspension was analyzed with a NICOMP model 370 Particle Size Analyzer. This instrument utilizes photon correlation spectroscopy for particle sizing in the submicron region. A small volume of the suspension was diluted with water and 5 placed in the cell of the particle size analyzer. Particle size determination based on volume weighted and number weighted particle size determination of the suspension, represented as a Gaussian distribution by the NICOMP 370 software, yielded the mean particle size values, which are listed below in Table I.

10

Table I: Volume-and Number-weighted Particle Size Stability of Microparticle-Cyclosporine

| 15 Time | Storage at 4°C | | Storage at 25°C | |
|------------|-------------------------|-------------------------|-----------------|-----------------|
| | Mean Particle Size (nm) | Mean Particle Size (nm) | Volume-Weighted | Number-Weighted |
| Days | Volume-Weighted | Number-Weighted | Volume-Weighted | Number-Weighted |
| 0 | 361 | 63 | 361 | 63 |
| 7 | 337 | 69 | 423 | 67 |
| 20 51 | 358 | 76 | 455 | 66 |

Approximately 20 µl of the freshly prepared suspension was placed on a clean slide, with a clean cover glass, and examined under 25 an Olympus BH2 microscope with 1000X magnification. An eye-piece equipped with a graticule was used to estimate the particle size. Most of the particles in the suspension were 0.3-0.5 µm.

Furthermore, microscopic examination of the suspension confirmed non-agglomerated or flocculated micron and sub-micron size drug particles exhibiting Brownian motion.

5

Example 2

For purpose of comparison (not according to the invention) using only a phospholipid, microparticle-cyclosporine with lecithin alone (without the second surface modifier, Tween 80) was also 10 prepared using the same procedure as Example 1. The suspension was stored in 20 ml glass vials for storage stability studies. The volume and number weighted mean particle size values of the suspension stored at 4 and 25°C are listed below. The results in 15 Table II illustrate that the presence of lecithin alone (without the presence of Tween 80) does not provide the particle size reduction and enhancement in storage stability as described in Example 1.

Table II: Volume-weighted Particle Size Stability of Microparticle-Cyclosporine

20

| Storage Time | Storage at 4°C | | Storage at 25°C | |
|--------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Mean Particle Size (nm) |
| Days | Volume-Weighted | Number-Weighted | Volume-Weighted | Number-Weighted |
| 0 | 704 | 91 | 704 | 91 |
| 1 | 1472 | 503 | 2230 | 755 |
| 6 | 1740 | 416 | 2290 | 874 |

Example 3

For purpose of comparison (not according to the invention) using only a surface modifier, microparticle-cyclosporine with Tween 80 alone (without a phospholipid, egg phosphatidylcholine) was also 5 prepared using the same procedure as Example 1. The suspension was stored in 20 ml glass vials. The results in Table III illustrate that the presence of Tween 80 alone (without the presence of phospholipid does not provide particle size reduction as in Example 1.

10 **Table III: Volume- and Number-weighted Particle Size
Stability of Microparticle-Cyclosporine**

| Mean Particle Size (nm) | | |
|-------------------------|-----------------|-----------------|
| Day | Volume-Weighted | Number-Weighted |
| 15 0 | 521 | 67 |

Example 4

The following microparticle-Docosanol formulations were prepared by the process of the invention with Tween 80, Tween 20, 20 egg phosphatidylcholine, and/or Phospholipon 90H as surface modifiers. Docosanol is available from Sigma. The formulations were prepared according to the procedures of Example 1. The compositions and concentration of excipients of the microparticle formulations are listed below:

Microparticle-Docosanol (Example 4.1, comparative)

| | |
|-------------------------|------------|
| Docosanol | 20 mg/ml |
| Egg Phosphatidylcholine | 50 mg/ml |
| Mannitol | 55 mg/ml |
| Distilled Water | qs to 100% |
| Total Volume | 20 ml |

Microparticle-Docosanol (Example 4.2)

| | | |
|----|-------------------------|------------|
| 10 | Docosanol | 20 mg/ml |
| | Egg Phosphatidylcholine | 50 mg/ml |
| | Mannitol | 55 mg/ml |
| | Tween 80 | 10 mg/ml |
| 15 | Distilled Water | qs to 100% |
| | Total Volume | 20 ml |

Microparticle-Docosanol (Example 4.3)

| | | |
|----|-------------------------|------------|
| 20 | Docosanol | 20 mg/ml |
| | Egg Phosphatidylcholine | 50 mg/ml |
| | Mannitol | 55 mg/ml |
| | Tween 20 | 10 mg/ml |
| 25 | Distilled Water | qs to 100% |
| | Total Volume | 20 ml |

Microparticle-Docosanol (Example 4.4)

| | | |
|---|------------------|------------|
| | Docosanol | 20 mg/ml |
| | Phospholipon 90H | 30 mg/ml |
| 5 | Mannitol | 55 mg/ml |
| | Tween 80 | 10 mg/ml |
| | Distilled Water | qs to 100% |
| | Total Volume | 20 ml |

10 Microparticle-Docosanol (Example 4.5, Comparative)

| | | |
|----|-----------------|------------|
| | Docosanol | 20 mg/ml |
| | Mannitol | 55 mg/ml |
| | Tween 80 | 10 mg/ml |
| 15 | Distilled Water | qs to 100% |
| | Total Volume | 20 ml |

The mean volume-and number-weighted particle size values of the suspension were 286 nm, and 98 nm, respectively.

20

The volume weighted mean particle size values of the above suspension stored at 4°C are listed below in Table IV.

**Table IV: Volume-weighted and Number Weighted
Particle Size Stability of Microparticle-Docosanol Stored at 4°C.**

| Storage Time | (Example 4.1) | | (Example 4.2) | |
|--------------|-------------------------|-----------------|-------------------------|-----------------|
| | Mean Particle Size (nm) | | Mean Particle Size (nm) | |
| Days | Volume-Weighted | Number-Weighted | Volume-Weighted | Number-Weighted |
| 0 | 688 | -- | 112 | 55 |
| 30 | ND | ND | 156 | 81 |

10

| Storage Time | (Example 4.3) | | (Example 4.4) | |
|--------------|-------------------------|-----------------|-------------------------|-----------------|
| | Mean Particle Size (nm) | | Mean Particle Size (nm) | |
| Days | Volume-Weighted | Number-Weighted | Volume-Weighted | Number-Weighted |
| 0 | 129 | 61 | 90 | 35 |
| 30 | 184 | 99 | 127 | 39 |

15

ND = Not Determined

20

The above data illustrate the much smaller particles produced by the present invention with the presence of a surfactant in addition to the phospholipid and that these particles retain their particle size over time without significant increase in size.

Example 5

The following seven microparticle-RTP-4055 (an antiviral drug) formulations were prepared with combinations of Tween 80,
5 Tetronic 908, Pluronic F-68, egg phosphatidylcholine, and/or phospholipon 90H as surface modifiers. The details of the sonication method are similar to those discussed in Example 1. The compositions and concentration of excipients of the microparticle formulations are listed below:

10

Microparticle-RTP-4055 (Example 5.1, Comparative)

| | | |
|----|-------------------------|------------|
| | RTP-4055 | 50 mg/ml |
| | Egg Phosphatidylcholine | 50 mg/ml |
| 15 | Distilled Water | qs to 100% |
| | Total Volume | 25 ml |

The mean volume weighted particle size of the suspension was 3195 nm.

20

Microparticle-RTP-4055 (Example 5.2)

| | | |
|----|-------------------------|------------|
| | RTP-4055 | 50 mg/ml |
| | Egg Phosphatidylcholine | 50 mg/ml |
| 25 | Mannitol | 55 mg/ml |
| | Pluronic F-68 | 5 mg/ml |
| | Distilled Water | qs to 100% |
| | Total Volume | 25 ml |

The mean volume- and number-weighted particle size values of the suspension were 672 nm and 76 nm respectively.

5 **Microparticle-RTP-4055 (Example 5.3)**

| | |
|-------------------------|------------|
| RTP-4055 | 50 mg/ml |
| Egg Phosphatidylcholine | 50 mg/ml |
| Mannitol | 55 mg/ml |
| 10 Tetronic 908 | 5 mg/ml |
| Distilled Water | qs to 100% |
| Total Volume | 25 ml |

15 The mean volume- and number- weighted particle size values of the suspension were 436 nm and 59 nm respectively.

Microparticle-RTP-4055 (Example 5.4, Comparative)

| | |
|---------------------|------------|
| RTP-4055 | 50 mg/ml |
| 20 Phospholipon 90H | 30 mg/ml |
| Distilled Water | qs to 100% |
| Total Volume | 25 ml |

25 The mean volume- number- weighted particle size values of the suspension were 1117 nm. and 108 nm respectively.

Microparticle-RTP-4055 (Example 5.5)

| | | |
|----|-------------------------|------------|
| | RTP-4055 | 50 mg/ml |
| | Phospholipon 90H | 30 mg/ml |
| 5 | Mannitol | 55 mg/ml |
| | Dimyristoylphosphatidyl | |
| | choline (DMPG) | 3 mg/ml |
| | Tween 80 | 10 mg/ml |
| | Distilled Water | qs to 100% |
| 10 | Total Volume | 25 ml |

The mean volume weighted particle size of the suspension was 236 nm. The particle size of the suspension stored at 4°C for 1 week and 1 month are 328 and 397 nm, respectively, which indicates the stability of the suspension.

Microparticle-RTP-4055 (Example 5.6)

| | | |
|----|------------------|------------|
| | RTP-4055 | 50 mg/ml |
| 20 | Phospholipon 90H | 30 mg/ml |
| | Mannitol | 55 mg/ml |
| | Tween 80 | 10 mg/ml |
| | Distilled Water | qs to 100% |
| | Total Volume | 25 ml |

25 The mean volume- and number- weighted particle size values of the suspension were 382 nm and 59 nm respectively. Within the

error limits, there was no variation in the mean particle size after one week of storage at 4°C.

Microparticle-RTP-4055 (Example 5.7, Comparative)

| | | |
|----|-----------------|------------|
| 5 | RTP-4055 | 50 mg/ml |
| | Mannitol | 55 mg/ml |
| | Tween 80 | 10 mg/ml |
| | Distilled Water | qs to 100% |
| 10 | Total Volume | 25 ml |

The volume- and number-weighted mean particle size values of the suspension were 545 nm, and 75 nm, respectively within the error limits, there was no variation in the mean particle size after one week
15 of storage at 4°C.

Example 6

20 The following six microparticle-Piroxicam formulations were prepared with combination of Tween 80, Tetronic 908, Pluronic F-68, and/or egg phosphatidylcholine as surface modifiers. Piroxicam was received from Cipla. The details of the sonication method are similar to those discussed in example 1. The compositions and concentration
25 of excipients of the microparticle formulations are listed below:

Microparticle-Piroxicam (Example 6.1)

| | |
|-------------------------|------------------|
| Piroxicam | 67 mg/ml |
| Egg Phosphatidylcholine | 67 mg/ml |
| 5 Mannitol | 67 mg/ml |
| Tween 80 | 5 mg/ml |
| Tetronic 908 | 5 mg/ml |
| Distilled Water | qs to 100% (w/v) |
| Total Volume | 15 ml |

10

The mean volume- and number- weighted particle size values of the suspension were 674 nm and 72 nm respectively.

Microparticle-Piroxicam (Example 6.2)

15

| | |
|-------------------------|------------------|
| Piroxicam | 67 mg/ml |
| Egg Phosphatidylcholine | 67 mg/ml |
| Mannitol | 67 mg/ml |
| Tetronic 908 | 5 mg/ml |
| 20 Distilled Water | qs to 100% (w/v) |
| Total Volume | 15 ml |

25 The mean volume- and number- weighted particle size values of the suspension were 455 nm and 58 nm respectively.

Microparticle-Piroxicam (Example 6.3)

| | | |
|---|-------------------------|------------------|
| | Piroxicam | 67 mg/ml |
| | Egg Phosphatidylcholine | 67 mg/ml |
| 5 | Mannitol | 67 mg/ml |
| | Pluronic F-68 | 5 mg/ml |
| | Distilled Water | qs to 100% (w/v) |
| | Total Volume | 15 ml |

10 The mean volume- and number- weighted particle size values of the suspension were 564 nm and 68 nm respectively.

Microparticle-Piroxicam (Example 6.4)

| | | |
|----|--------------------------------|------------------|
| 15 | Piroxicam | 67 mg/ml |
| | Egg Phosphatidylcholine | 67 mg/ml |
| | Mannitol | 67 mg/ml |
| | Tween 80 | 5 mg/ml |
| 20 | Cetyltrimethylammonium bromide | 10 mg/ml |
| | Distilled Water | qs to 100% (w/v) |
| | Total Volume | 15 ml |

25 The mean volume- and number- weighted particle size values of the suspension were 479 nm and 80 nm respectively.

Microparticle-Piroxicam (Example 6.5)

| | | |
|----|--------------------------------|------------------|
| | Piroxicam | 67 mg/ml |
| 5 | Egg Phosphatidylcholine | 67 mg/ml |
| | Mannitol | 67 mg/ml |
| | Cetyltrimethylammonium bromide | 10 mg/ml |
| 10 | Distilled Water | qs to 100% (w/v) |
| | Total Volume | 15 ml |

The mean volume- and number- weighted particle size values of the suspension were 670 nm and 128 nm respectively.

15 Microparticle-Piroxicam (Example 6.6, Comparative)

| | | |
|----|-----------------|------------|
| | Piroxicam | 67 mg/ml |
| 20 | Mannitol | 67 mg/ml |
| | Tween 80 | 5 mg/ml |
| | Tetronic 908 | 5 mg/ml |
| | Distilled Water | qs to 100% |
| | Total Volume | 25 ml |

25 The volume- and number- weighted particle size values of the suspension were 1184 nm and 385 nm, respectively.

WHAT IS CLAIMED IS:

- 3 1. A composition of microparticles of a water-insoluble
4 substance comprising particles of an industrially useful water-
5 insoluble or poorly soluble compound, a phospholipid and at least one
6 non-ionic, anionic or cationic surfactant, in which the surfactant or
7 surfactants provide volume-weighted mean particle size values of the
8 water-insoluble compound at least 50% smaller than particles
9 produced without the presence of the surfactant using the same energy
10 input.

- 1 2. A pharmaceutical composition of microparticles of a water-
2 insoluble substance comprising particles of an industrially useful
3 water-insoluble or poorly soluble compound, a phospholipid and at
4 least one non-ionic, anionic or cationic surfactant, in which the
5 surfactant or surfactants provide volume-weighted mean particle size
6 values of the water-insoluble compound at least 50% smaller than
7 particles produced without the presence of the surfactant using the
8 same energy input.

- 1 3. The pharmaceutical composition of claim 2 for oral,
2 inhalation, ocular, nasal or injectable administration.

- 1 4. The pharmaceutical composition of claim 3 in injectable
2 form for intravenous, intra-arterial, intra-muscular, intradermal,
3 subcutaneous, intra-articular, cerebrospinal, epidural, intracostal,
4 intraperitoneal, intratumor, intrabladder, intra-lesion or
5 subconjunctival administration.

1 5. A dried suspension of the composition of claim 4 which can
2 be resuspended in aqueous or non-aqueous media.

1 6. A suspension, spray-dried powder, lyophilized powder
2 granules or tablets of the composition of claim 2.

1 7. A composition of claim 1 in which the water-insoluble
2 compound is a biologically useful compound or an imaging agent.

1 8. The composition of claim 1 or claim 2 wherein the
2 surfactant is a polyoxyethylene sorbitan fatty acid ester, a block
3 copolymer of ethylene oxide and propylene oxide, a tetrafunctional
4 block copolymer derived from sequential addition of ethylene oxide
5 and propylene oxide to ethylenediamine, an alkyl aryl polyether
6 sulfonate, polyethylene glycol, hydroxy propylmethylcellulose,
7 sodium dodecylsulfate, sodium deoxycholate,
8 cetyltrimethylammonium bromide or combinations thereof.

1 9. The process of claim 1 or 2 wherein the phospholipid is of
2 egg or plant origin or semisynthetic or synthetic in partly or fully
3 hydrogenated form or in a desalted or salt form such as
4 phosphatidylcholine, phospholipon 90H or dimyristoyl
5 phosphatidylglycerol sodium salt, phosphatidylethanolamine,
6 phosphatidylserine, phosphatidic acid, lysophospholipids or
7 combinations thereof.

1 10. A process for preparing sub-micron and micron sized,
2 stable particles of water-insoluble or a poorly soluble industrially
3 useful compound using natural or synthetic phospholipids, said
4 process comprising reducing the particle size by sonication,
5 homogenization, milling, microfluidization and precipitation, or
6 recrystallization and precipitation of the compound using antisolvent
7 and solvent precipitation including from supercritical fluids in the
8 presence of a phospholipid and at least one non-ionic, anionic or
9 cationic surfactant.

1 11. A process of preparing microparticles of a water-insoluble
2 or poorly soluble compound comprising the steps of:
3 (1) mixing particles of a water-insoluble or poorly soluble
4 industrially useful compound with a phospholipid and at least one
5 non-ionic, anionic or cationic surfactant, and thereafter
6 (2) applying energy to the mixture sufficient to produce
7 volume-weighted mean particle size values of the compound at least
8 50% smaller than particles produced without the presence of the
9 surfactant using the same energy input.

1 12. The process of claim 10 or 11 wherein the phospholipid is
2 of egg or plant origin or semisynthetic or synthetic in partly or fully
3 hydrogenated form or in a desalted or salt form such as
4 phosphatidylcholine, phospholipon 90H or dimyristoyl
5 phosphatidylglycerol sodium, salt, phosphatidylethanolamine,
6 phosphatidylserine, phosphatidic acid, lysophospholipids, or
7 combinations thereof.

1 13. The process of claim 10 or 11 wherein the surfactant is a
2 polyoxyethylene sorbitan fatty acid ester, a block copolymer of
3 ethylene oxide and propylene oxide, a tetrafunctional block
4 copolymer derived from sequential addition of ethylene oxide and
5 propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate,
6 polyethylene glycol, hydroxy propylmethylcellulose, sodium
7 dodecylsulfate, sodium deoxycholate, cetyltrimethylammonium
8 bromide or combinations thereof.

1 14. The process of claim 10 or 11 wherein the surfactant is
2 present above the critical micelle concentration.

1 15. The process of claim 10 or 11 in which the compound is a
2 biologically useful compound or an imaging agent.

1 16. A composition comprising microparticles prepared by the
2 process of claim 10.

1 17. A composition comprising microparticles produced by the
2 process of claim 11.

INTERNATIONAL SEARCH REPORT

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| International Application No PCT/US 97/04695 |
|---|

| A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K9/51 A61K9/14 A61K49/04 | | |
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| According to International Patent Classification (IPC) or to both national classification and IPC | | |
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| Electronic data base consulted during the international search (name of data base and, where practical, search terms used) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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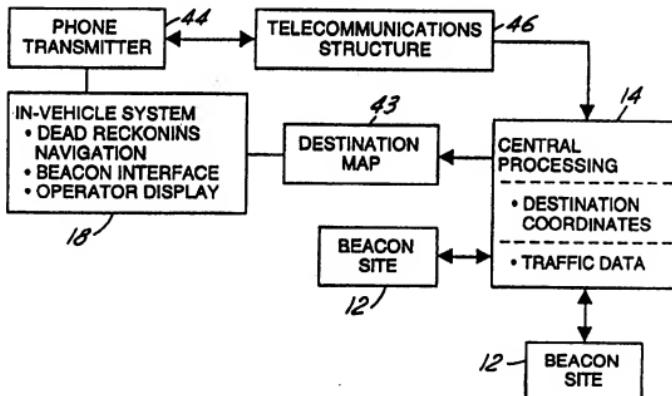


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| (72) Inventors: SULICH, Janusz, S.; 20919 McClung Avenue, Southfield, MI 48075 (US); KNOCKEART, Ronald, P.; 848 Shrewsbury, Clarkston, MI 48348 (US). | | |
| (74) Agent: WELLS, Russel, C.; Siemens Automotive Corporation, 2400 Executive Hills Drive, Auburn Hills, MI 48326-2980 (US). | | |

(54) Title: VEHICLE NAVIGATION AND ROUTE GUIDANCE SYSTEM



(57) Abstract

An IVHS equipped vehicle utilizes telecommunication means (46) for determining and loading geodetic coordinates of its destination location into the information processor in the invehicle unit (18). The operator merely dials the phone number of his/her destination on the keyboard (28) in the vehicle and the system operates to transmit the phone number to a central processing means (14) and data base (16) where the phone number is converted to the geodetic coordinates which are transmitted back to the invehicle system.

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Vehicle Navigation and Route Guidance System

This invention relates to data processing control systems in general and more particularly to motor vehicle navigation and route 5 guidance systems determining the destination address from a telephone number. US class 364/444 or int. class G06F 15/50.

BRIEF SUMMARY OF THE INVENTION**Background of the Invention**

10 There are fundamentally two different types of vehicle navigational systems. The first system makes use of stored map displays wherein the maps of a predetermined area are stored in the invehicle computer and displayed to the vehicle operator or driver. The maps, knowing the location where the vehicle started and where it is 15 to go, will highlight the direction and the driver will have to read the display and follow the route. One such stored map display system offered by General Motors on their 1994 Oldsmobile, uses Global Positioning System (GPS) satellites and advanced dead reckoning techniques to determine a precise location. The driver enters details of 20 the desired destination into an on-board or invehicle, computer, in the form of specific address, a road intersection, etc. The stored map is displayed and the operator then pinpoints the desired destination. The on-board computer then calculates the most efficient route. The on-board computer then displays on a display unit, the distance to and the 25 direction of each turning maneuver in easy-to-read graphics and also includes a voice prompt.

The second system, such as the Siemens Ali-Scout™ system, requires that the driver key-in the destination address, in geodetic 30 coordinates, into the invehicle computer. A compass means located in the vehicle then gives a "compass" direction to the destination address. Such a "compass" direction is shown in easy-to-read graphics as an arrow on a display unit indicating the direction the driver should go. Along the side of the road are several infrared beacon sites which 35 transmit data information to the properly equipped vehicle relative to

the preferred routing to the next adjacent beacon sites. From all of the data the invehicle computer receives, the invehicle computer selects the desired beacon data information to the next beacon along the route direction to the final destination and displays a graphic symbol for the

- 5 vehicle operator to follow and the distance to the desired destination. There is no map to read, only a simple graphic symbol and a voice prompt telling the vehicle operator where to turn and when to continue in the same direction.

- 10 U.S. Patent 4,350,970, assigned to Siemens AG and issued on September 21, 1982 to von Tomkewitsch and entitled "Method for Traffic Determination in a Routing and Information System for Individual Motor Vehicle Traffic" describes a method for traffic management in a routing and information system for motor vehicle
15 traffic. The system has a network of stationary routing stations, each located in the vicinity of the roadway, which transmit route information and local information concerning its position to passing vehicles. The route information which is transmitted is the preferred routing to all beacons and zones adjacent to the beacon site. The
20 vehicle navigation system then selects a route from all the routes transmitted by the beacon.

- The trip destination address, via geodetic coordinates, is loaded by the vehicle operator into an onboard device, a navigation processor,
25 in the vehicle and by dead reckoning techniques a distance and direction graphic is displayed. The first routing station which the vehicle passes transmits a message to the vehicle with route data to all of the adjacent beacons one of which is the next routing station. The vehicle receives the message and selects one of the recommended
30 routes which will guide the vehicle towards its final destination. As it executes the travel to the next beacon, it accumulates time and distance traveled which it transmits to the second routing station when it is interrogated by passing the second routing station. In this manner, traffic management is updated in real time and the vehicles
35 are always routed the "best way". Of course the best way may be the

shortest way, the less traveled way, the cheapest way or any combination of these plus other criteria.

Summary of the Invention

- 5 In both of the aforementioned systems, the vehicle operator has had to enter into the invehicle computer, the geodetic coordinates of the destination address. These are latitude and longitude coordinates. In each case, the present systems require each coordinate to be at least a six digit number, degrees, minutes, and seconds; thus, two six
10 10 digit numbers must be entered. In order to get the coordinates, the vehicle operator has to read a map or consult a look-up table and by means of a data keyboard, key in the numbers.

- In the Ali-Scout system, these coordinates would be inputted
15 15 into the navigation computer and until the vehicle passed the first beacon site, the vehicle display system indicates the compass heading to take. Once the vehicle passed the first beacon, the vehicle will then receive information about the best route to take to the next adjacent beacons and the computer, knowing its present geodetic location and
20 20 the geodetic location of its destination address, will select the best route in the direction of the destination address.

- One method of introducing the coordinates of the destination address requires the vehicle operator to study a map, a manual or
25 25 some other data base means to determine the six digit word coordinates of the destination address and then enter or key in each word by means of a keyboard into the onboard memory. In the present invention, the vehicle operator enters the phone number of the destination by means of a phone-type number keyboard pad. The
30 30 onboard computer transmits this number to the central processing station having a data base subsystem wherein the correlation of the phone number, physical address and the geodetic coordinates such as latitude and longitude are stored. The central processor then transmits the geodetic coordinates back to the onboard vehicle computer and the

destination address is automatically and correctly loaded into the onboard memory.

In addition, the invention herein provides for the entry of data
5 input from various traffic functions to be put in the data base and such information is transmitted to the various beacon sites in the system by the central processing unit to provide better data to the vehicles concerning routing vectors.

10 It is therefore a principle advantage to have the operator load the destination address into the invehicle system by the simple means of dialing the telephone number of the destination and not requiring the operator to refer to various maps or other data bases for such destination coordinates.

15 20 It is another advantage to have the beacon sites be updated with traffic functional information such as travel conditions and road data, received from many sources and inputted into the central processing unit and which is transmitted to the beacon sites from the system central processing unit.

These and other advantages will become apparent from the following drawings and detailed description.

25 **Brief Description of the Drawings**

In the drawings:

FIG. 1. is a system block diagram of the vehicle navigation and route guidance system;

30 FIG. 2 is a detailed block diagram of the preferred embodiment of the invention;

FIG. 3 is a driver interface device in the vehicle for transmitting the destination phone number and accepting or rejecting a system recommended route.;

FIG. 4 is a dashboard display in the vehicle to receive one form of the information from the navigation unit including a preview of the routing and alert by the system of an in-route change of routing;

5 Detailed Description of the Preferred Embodiment

Referring to FIG. 1 by the characters of reference, there is illustrated in block diagrammatic form a vehicle navigational and routing system 10. As is known in the prior art, there are located along the sides of the roadway, several beacon sites 12, or fixed guide 10 beacons 13, each placed strategically in the area. In an urban area, such sites are within kilometers of each other and in the rural areas, the beacon sites maybe spaced much, much farther apart.

It is the function of the beacon sites 12 to transmit information 15 received from a central processing means 14 including an information processor 15 and a data base 16 concerning the best route to take to the adjacent beacon sites. The beacon sites 12 also receive information from a vehicle including among other information about the elapsed travel time for links vehicle activity and other information to 20 assist in determining the "best" route from that particular beacon site 12 to each adjacent beacon site 12 and beyond.

In the prior art systems, such as the Ali-ScoutTM System as developed by Siemens Aktiengesellschaft, the vehicle operator has, in 25 his vehicle, an invehicle system 18 comprising a transmitter 20, a receiver 22, an information processing unit 24 including a position unit, a travel-time unit, a destination address processor and a memory and a display means 26 having a data entry means such as a keyboard 28, an arrow indicator guidance visual display 30 and an audio or 30 voice messaging system 32. The Ali-ScoutTM System is an infrared communication system in that the medium for transmitting and receive data from a beacon 12 to the invehicle system 18 is by means of infrared waves.

- 6 -

In the prior art system, the vehicle operator had to input the particular geodetic destination address coordinates found on a map or some other data look-up means. These coordinates are typically the latitude and longitude of the destination address which, of necessity, 5 are long digital words. The operator must accurately read these numbers from his map and key them into the invehicle unit through the alpha numeric keyboard.

In the preferred embodiment, the operator 34 by knowing the 10 phone number of his destination location does not have to read and copy unfamiliar numbers into a keyboard. The typical phone number in the United States is 7 digits long, not including the area code, and is probably a familiar number. Therefore, the error of entering such a number is much smaller. The invehicle system 18 transmits the phone 15 number to the central processor 14 and data base 16 as a destination address request by conventional telecommunications methods such as a cellular telephone network or spread spectrum telephone network. The central processor 14 and data base 16, has the street address and the geodetic coordinates corresponding to the phone number stored in the 20 data base 16 and coded in the proper form. This coded form is then transmitted from the central processor 14 to the invehicle unit 18 as a destination location address. Once the coordinates are located in the invehicle unit 18, the system then prepares to receive from the next 25 beacon site 12 which it passes, the route information to the next adjacent beacon sites 12.

Another feature of the preferred embodiment is the information gathering capabilities of the central processor 14 and data base 16. The information gathered is received from other sources such as 30 special events data 36 regarding such as sporting or cultural events, traffic events data 38 regarding such as accidents and road repairs, weather data 40, and other transient and incident data 42 which would affect the movement of vehicles along the highways and streets.

It is understood that the transmitters and receivers in the invehicle unit 18, the central processor 14 and the beacon sites 12, function to communicate and receive data between and among the several units as the IVHS system requires. As will be shown, the 5 transmitter 20 can transmit data in the communication mode to the central process 14 and later communicate in the infrared range to the beacon sites 12.

Referring to FIG. 2, there is illustrated in block diagrammatic 10 form, the preferred embodiment of the system of FIG. 1. FIG. 2 is a less detail block diagram which shows the invehicle system containing the dead reckoning navigational system which is a basic element of the system and the destination map 43 which is stored in the invehicle system. The dead reckoning navigational system gives the operator 34 15 a compass bearing on the direction to go to the destination location. In addition, the dead reckoning system maintains the proper compass headings in the vehicle in order to accurately show the direction the vehicle must travel to reach its destination location when the vehicle is in the autonomous mode of operation, that is before intercepting its 20 first beacon site 12 or when off the course derived from the beacon. Also the invehicle system 18 has the beacon interface which was previously described incorporating an infrared communication system. The operator display means 26 was also previous described and will be described in alternate embodiments with regard to FIGs. 3 and 4.

25 The new feature of the preferred embodiment is the phone transmitter 44 which allows the destination location telephone number to be transmitted through the telecommunications structure or communications means 46 to the central processor 14. At the central 30 processor 14, the destination location or address telephone number is converted to geodetic or map coordinates and transmitted by the communications means 46 to the invehicle system 18. In the alternative, the central processor 14 can also identify a specific street address corresponding to the dialed telephone number. This 35 information can also be used for navigational purposes, or as additional

information for the operator. By means of the information provided by the beacon site 12, the invehicle system 18 then operates to guide the vehicle from its present position or location to its destination address or location. In addition as previously indicated, the central processor 5 14 also transmits preferred routes to the beacon sites 12 based on its knowledge of area-wide traffic conditions.

- Once the geodetic coordinates from the central processor 14 are inputted into the in vehicle system 18 the invehicle information processing unit 24 takes the information from its memory as to its present location, generates a direction indicator on the visual display screen 30 directing the vehicle operator 34 as to the direction to go. Once the vehicle is in position to communicate and does communicate with the first beacon site 12, the information processing unit 24 10 selects the "best" route using the beacon site 12 supplied information. The beacon site 12 transmits information on how to go to each adjacent beacon site 12 and it is the function of the invehicle information processing unit 24 is to select the appropriate direction information knowing its present location and the destination location. 15 20 All other information received from the beacon site 12 may not utilized. The routing is either audibly 32 or visually displayed 30 or both to the vehicle operator 34 and is updated each time the vehicle is instructed to change course. The beacon site 12 receives information from the invehicle information processing unit 24 as to the amount of time and distance the car has traveled from a previous beacon so as to update the central processor 14 and data base 16 for potential new routing information. At no time does any part of the system, other than the invehicle system 18 know where the vehicle is and where it is going. This preserves the anonymity of each vehicle. 25

- 30 In FIG. 3, there is illustrated a keyboard 28 having a numeric keypad 48 similar to that found on a telephone. This promotes ease of data entry since most are familiar with a touch-tone phone keyboard. Since this is a telephone, there are selection buttons 50 and 52 which 35 allow the operator 34 to indicate that the number being indexed into

the keypad 48 is either for telecommunications 50 or for IVHS communications 52. As with most telephone keypads, the number entered into the keypad 48 is displayed on a display panel 54 before it transmitted. Once the correct number is displayed, the operator 5 confirms and activates the telephone. When the destination address coordinates are returned to the invehicle system 18, the operator 34 indicates his or her acceptance by pushing the accept route button 56 and the vehicle is now able to function in the IVHS mode.

10 As soon as telecommunications such as a cellular phone is used, total anonymity is no longer available. Location of a vehicle can be established from the transmissions, but this is only implementable by the central processor 14 having total access to the communications network. As to the message content, the destination and routing could 15 be overheard, but the location and identification of the receiving vehicle would not be available to a casual listener. For example, a scanner will pick-up the data transmission, but not the location of the receiving vehicle or person.

20 One such method of communicating the route to the vehicle operator is by a visual display device 58 as illustrated in FIG. 4. This is intended to show a different mode of visual display to the operator 34; i.e. after the invehicle information processing means 24 selects the 25 routing from the data received from the beacon 12, it can display the routing in the following manner.

In this example, the visual display 30 shows the several street names 60 that the vehicle will take to get to its destination location or address. Next to each street name 60 is the compass direction 62 the 30 vehicle should proceed on that particular street 60. Both the route time 64 and the route mileage 66 is or maybe shown to the vehicle operator 34.

Of key importance, is the data signal from the central processor 35 14 and data bank 16 to the beacon sites 12 that alerts the beacon

sites to a change in the route because of information gathered by the system from the several beacons or other input means. Such information may show heavy traffic congestion or a sudden repair problem such as a water main break. This information is transmitted
5 by the central processor 14 and data base 16 to the site computer 68 at each beacon site 12 to alter the routes from each beacon site to the adjacent beacon sites. The end result may be to redirect the vehicle when it passes the next beacon site and thereby changing the display. The visual display device 58 may have a display 70 which alters the
10 operator 34 to a route change.

Still other embodiments of the system may provide information in a package form to commercial vehicles such as trucks. In this case, which is an example of a dedicated system, the anonymity is not an issue. The beacons are either owned or operated by the trucking company, or if there is a consortium of several companies, data from
15 each company can be encrypted.

The dispatching department of the a freight company can
20 access the central processor and data base with a routing for a given truck that is entering the area. This routing coincides with the delivery points where the truck is to stop and discharge its load or a partial load. As an example, ABC Cartage Company knows that its truck, having a particular identification, will be arriving in the area with a load
25 of goods that is to be delivered to five different stops. The dispatching or similar department enters the destination address information of the different stops into the central processor. When the beacon site picks up the truck for the first time, it pulls the information of the five stops from the data bank and transmits that information to the invehicle
30 information processing means 24 as a destination address message. The invehicle system 18 processes the best route for the driver from the normal beacon information. In the alternative, if the dispatcher deems the order of the stops is important because of the vehicle loading, the dispatcher develops the required destination address

message and adds any other information so that the driver follows the best route.

There has thus been described an IVHS system wherein the
5 geodetic coordinates of the destination location are transmitted over a
communications system to the invehicle system by means of standard
telephone communications. The telephone number of the destination
location is transmitted from the vehicle operator 34 to a central
processor 14 where it addresses a data base 16 to extract the
10 geodetic coordinates of the location having that phone number. The
central processor 14 then transmits those coordinates via the
communications medium to the invehicle information processing
means 24. Thus, the information processing means 24 develops the
15 direction that the vehicle is to take from its present location to the
destination with information being received from the several beacon
sites 12 which the vehicle passes.

We Claim:

1. In a vehicle navigation system for directing a vehicle from its present location to a destination location having

an invehicle system with a keyboard means adapted to receive the geodetic coordinates of the destination location of the vehicle from the vehicle operator;

5 information processing means operatively connected to said keyboard means for processing the geodetic coordinates of the destination location to generate a route for the vehicle to travel from its present location to the destination location, said information processing means having the present location geodetic coordinates,

10 display means responding to the information processing means for audibly and visually displaying route information to the destination location;

15 beacon means positioned at predetermined intervals along the roadways on which the vehicle travels, said beacon means adapted to transmit and receive navigational data to said invehicle system; said beacon means adapted to receive information from a central processing means;

20 central processing means including an information processor adapted to receive input data from said beacon means and to generate updated routing data and for transmitting said updated navigational data to said beacon means;

the improvement comprising:
25 communications means in the vehicle responsive to a telephone number inputted in the keyboard means identifying the destination location, said communication means operable to transmit said telephone number to the central processing means;

30 receiving means in the central processing means for receiving said telephone number;

said information processing means operatively connected to said receiving means for generating geodetic coordinates corresponding to said telephone number and for transmitting said geodetic coordinates

35 to said invehicle system for processing by the invehicle information processing means.

2. In a vehicle navigation system according to claim 1, wherein said communication means is a cellular telephone network.

3. In a vehicle navigation system according to claim 1, wherein said communication means is a wireless communications telephone network.

4. In a vehicle navigation system for directing a vehicle from its present location to a destination location having

5 an invehicle system with a keyboard means adapted to receive the geodetic coordinates of the destination location of the vehicle from the vehicle operator,

10 information processing means operatively connected to said keyboard means for processing the geodetic coordinates of the destination to generate a route for the vehicle to travel from its present location to the destination location, said information processing means having the present location geodetic coordinates,

15 display means responding to the information processing means for audibly and visually displaying route information to the destination location;

20 beacon means positioned at predetermined intervals along the roadways on which the vehicle travels, said beacon means adapted to transmit and receive navigational data to said invehicle system; said beacon means adapted to receive information from a central processing means;

25 central processing means including an information processor adapted to receive input data from said beacon means and to generate updated routing data and for transmitting said updated navigational data to said beacon means;

the improvement comprising:

25 said central processing means having input means for receiving traffic data and information from several sources relating to vehicle travel for generating updated navigational data; said central processing means also having transmitting means for transmitting said updated navigational data to said beacon means.

5. In a vehicle navigation system according to claim 4 wherein in said central processing means one of said sources responds to weather data and another of said sources responds road condition data and said central processing means generates said updated navigation data including weather and road conditions to said beacon means for transmission to the invehicle systems.

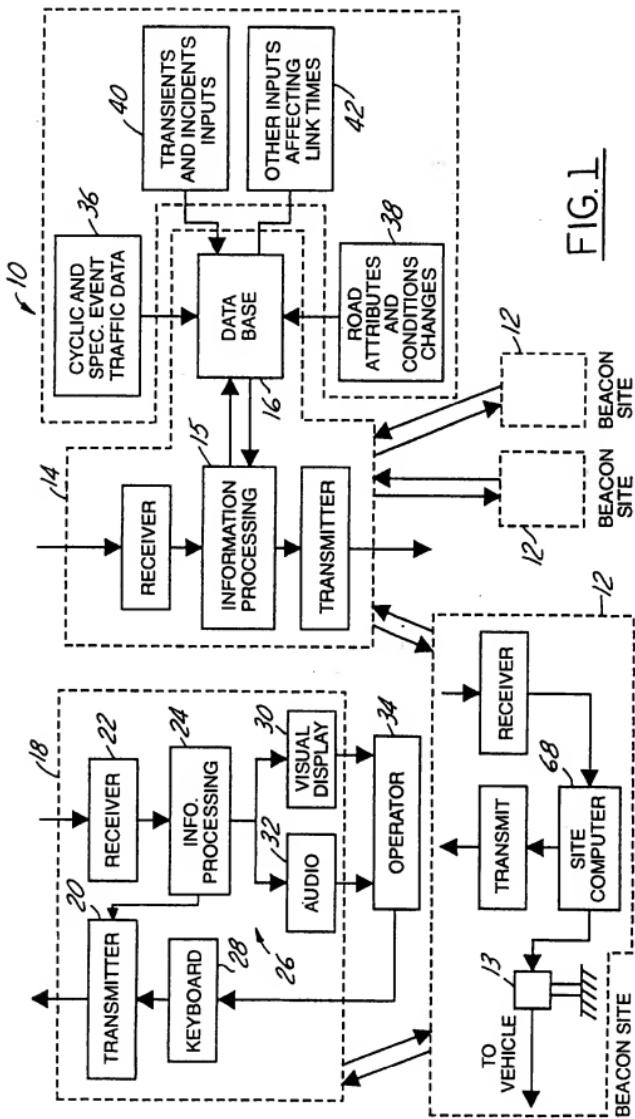
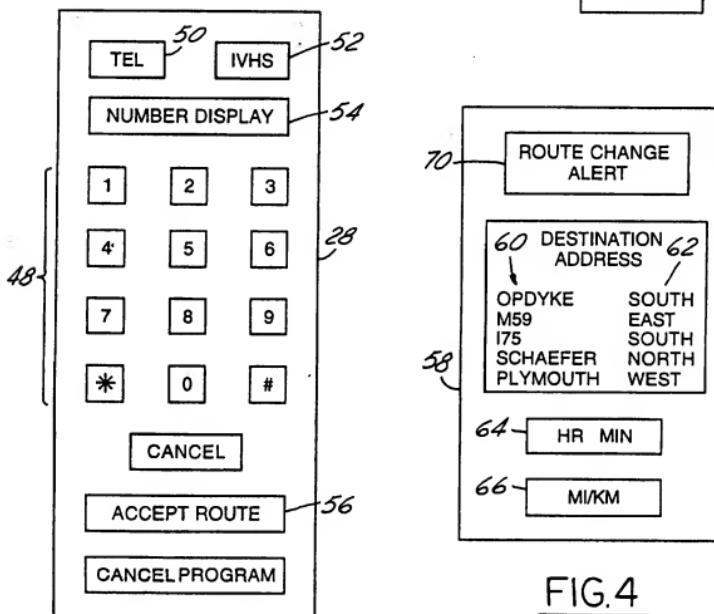
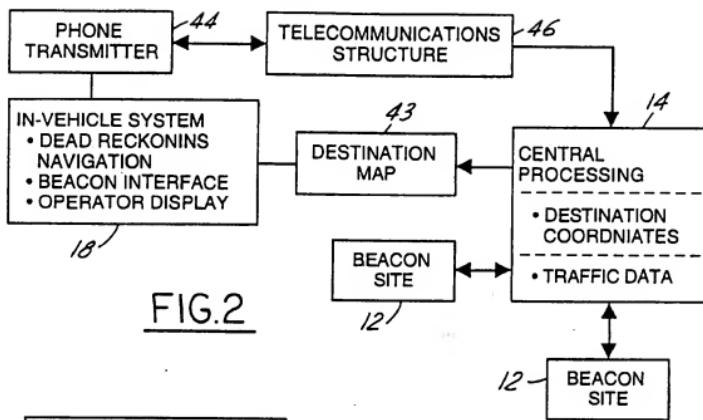


FIG. 1

FIG. 3FIG. 4

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(54) Title: PHARMACEUTICAL SUSPENSION COMPOSITIONS LACKING A POLYMERIC SUSPENDING AGENT

(57) Abstract: Stable aqueous pharmaceutical suspension compositions containing lecithin as a stabilizing additive and lacking a polymeric suspending agent are disclosed.

**PHARMACEUTICAL SUSPENSION COMPOSITIONS LACKING A
POLYMERIC SUSPENDING AGENT**

5 1. Background of the Invention

The present invention relates to pharmaceutical suspension compositions. In particular, this invention relates to physically stable aqueous pharmaceutical compositions of water-insoluble drugs.

10

2. Description Of Related Art

Aqueous pharmaceutical suspension compositions typically contain one or more polymeric suspending or viscosity-enhancing agents to enhance 15 physical stability. The polymeric suspending agents, which can be ionic or nonionic, help keep the water-insoluble components of the composition suspended. The polymeric suspending agents also make it easier to resuspend the composition after water-insoluble components have settled to the bottom of a container.

20

Many polymeric suspending agents are known. Polymeric suspending agents commonly used in aqueous pharmaceutical suspension compositions include carbomers, polyvinyl alcohol, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, 25 methyl cellulose, carboxymethyl cellulose, microcrystalline cellulose, powdered cellulose, xanthan gum, gellan gum, carageenan, acacia, tragacanth, gelatin, guar gum, alginic acid, sodium alginates, propylene glycol alginate, eudragit (methacrylic acid and methyl methacrylate copolymer), dextrin, dextran, dextran-polyethylene glycol conjugates, and the glycosaminoglycans family of 30 polymers, such as heparin sulfate, heparan sulfate, dermatan sulfate, chondroitin sulfate.

U.S. Patent No. 5,843,930 discloses topically administrable ophthalmic and otic compositions comprising (a) ciprofloxacin in aqueous solution in an amount effective for antibacterial action; (b) a non-ionic viscosity augmenter unaffected by pH and ionic level, said viscosity augmenter being present in an
5 amount effective for augmenting the viscosity of the composition to a viscosity greater than that of water, said viscosity augmenter being at least 85% hydrolyzed polyvinyl alcohol; (c) a non-ototoxic preservative present in an amount effective for antibacterial action the preservative being benzyl alcohol; (d) water sufficient to produce an aqueous composition; (e) hydrocortisone in
10 aqueous suspension in an amount effective for anti-inflammatory action; (f) lecithin in an amount effective for enhancing suspension of other constituents in the compositions; and (g) polysorbate ranging from polysorbate 20 to 80 in an amount effective for spreading the preparation on a hydrophobic skin surface to the site of infection or inflammation.

15

According to the '930 patent, the compositions comprising ciprofloxacin and hydrocortisone contain polyvinyl alcohol in an amount effective for augmenting the viscosity of the composition to a viscosity greater than that of water and suspending other constituents of the composition. To allow a
20 ciprofloxacin preparation to be administered in drops from a medicine dropper and to flow by gravity to and remain or deposit in an effective amount at a selected area, a viscosity-augmenting agent that would also serve to suspend hydrocortisone was desirable. For compatibility with ciprofloxacin hydrochloride solubility, viscosity-augmenting agents were preferably non-ionic and
25 unaffected by pH and ionic level. See Col., 8, lines 13-31 of the '930 patent.

Polyvinyl alcohol was selected for its ability to produce a suitable viscosity and a high ability to suspend hydrocortisone in aqueous preparations. See the '930 patent at Col. 8, lines 32-37. The addition of lecithin to the
30 composition enhanced the efficacy of polyvinyl alcohol in suspending hydrocortisone in aqueous preparations with ciprofloxacin hydrochloride and other components. See the '930 patent at Col. 8, line 64 – Col. 9, line 12.

The '930 patent discloses a process for manufacturing compositions containing ciprofloxacin and hydrocortisone in Example 5 at Column 5, lines 27-67. According this manufacturing process, polyvinyl alcohol, lecithin, benzyl alcohol and acetic acid are sequentially added to prepare a first stock solution. Separately sodium chloride and sodium acetate are dissolved in water to form a second stock solution. A third stock solution is prepared by dissolving polysorbate 20 and dispersing hydrocortisone in water. Finally, ciprofloxacin is either added to the first stock solution or ciprofloxacin is prepared as a fourth stock solution by dissolving ciprofloxacin, acetic acid and sodium acetate to form a ciprofloxacin stock solution. After the first and second stock solutions are combined, the ciprofloxacin stock solution is added to the combined solution. Finally, the third stock solution polysorbate 20 and hydrocortisone is mixed with the remaining batch volume.

15

A suspension composition's physical stability can be measured by two common methods. First, the resuspendability of a composition can be measured by allowing a homogeneous to remain standing in a cylindrical container for a period of time, then determining the number of inversions of the cylindrical container necessary to resuspend any sediment that form while the composition was standing. Second, the rate of settling can be measured by allowing a homogeneous suspension composition to remain standing for a period of time, then observing the height of sedimentation visible in a sample contained in a cylinder. Larger sedimentation heights indicate less separation with less supernatant liquid. Both measures of physical stability are important. A composition that is very easy to redisperse but that settles too quickly can be difficult to manufacture. Suspension compositions must remain well dispersed during processing and filling operations while commercial supplies are prepared in order to insure uniform products.

Summary Of The Invention

The present invention provides aqueous pharmaceutical suspension compositions that have excellent physical stability. The compositions contain one or more drugs that are insoluble or sparingly soluble in water such that at least a portion of the drug compound(s) contained in the compositions of the present invention is intended to be suspended. The compositions contain a physical-stability enhancing additive consisting essentially of lecithin.

The present invention also relates to a method of preparing an aqueous pharmaceutical suspension composition comprising lecithin but lacking a polymeric suspending agent. According to the present invention, a water-insoluble drug compound is mixed in a lecithin dispersion prior to being combined with the balance of the aqueous suspension composition.

Among other factors, the present invention is based upon the finding that a specific order of addition of ingredients in compositions containing a water-insoluble drug and lecithin but lacking a polymeric suspending agent provides such compositions with excellent physical stability. Compositions prepared by dispersing a water-insoluble drug with lecithin prior to mixing the drug with the balance of ingredients in the compositions have superior physical stability compared to those prepared by combining all ingredients in one step or by dispersing the water-insoluble drug with only a surfactant prior to mixing the drug with the balance of the composition.

Detailed Description Of The Invention

Unless otherwise indicated, all ingredient concentrations are listed as percent (w/w).

As used herein, "water-insoluble drug compound" means a drug compound that is insoluble or poorly soluble in water such that in the final

pharmaceutical composition at least a portion of the total amount of the drug compound is intended to be in suspension rather than in solution.

As used herein, "physical-stability enhancing additive consisting essentially of lecithin" means that the suspension composition contains lecithin but lacks a polymeric suspending agent or polymeric viscosity-enhancing agent. Typical polymeric suspending agents or polymeric viscosity-enhancing agents include carbomers, polyvinyl alcohol, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose, carboxymethyl cellulose, microcrystalline cellulose, powdered cellulose, xanthan gum, gellan gum, carageenan, acacia, tragacanth, gelatin, guar gum, alginic acid, sodium alginates, propylene glycol alginate, eudragit (methacrylic acid and methyl methacrylate copolymer), dextrin, dextran, dextran-polyethylene glycol conjugates, and the glycosaminoglycans family of polymers, such as heparin sulfate, heparan sulfate, dermatan sulfate, chondroitin sulfate.

The compositions of the present invention contain a therapeutic or prophylactic amount of one or more water-insoluble drug compounds. The amount of such water-insoluble drug compounds depends on a number of factors including individual drug potency, targeted indication, etc. Typical drug concentrations range from about 0.001 – 5%. Many water-insoluble drugs are known, including steroids such as dexamethasone; rimexolone; prednisolone; hydrocortisone; fluticasone propionate; budesonide; mometasone furoate monohydrate; and dexamethasone becloxil. Water-insoluble compounds other than steroids include griseofulvin; carbamazepin; clofibrate; ketoprofen; 5-flurouracil; flurbiprofen; mefanamic acid; flufenamic acid; and crystalline beta escincic acid.

Particularly for topical ophthalmic use, small particle sizes of the water-insoluble drug are preferred. As used herein, "micronized" drug particles means drug particles having an average particle size $\leq 10 \mu\text{m}$ (based on

surface area (dsn)). If the particle size of the drug raw material as received from the supplier is unsatisfactory, one or more known sizing techniques, such as ball milling or micronizing, can be used to adjust the particle size into the desired range.

5

To enhance the physical stability of the suspension composition of present invention, the composition contains a physical-stability enhancing additive consisting essentially of lecithin or a lecithin derivative. Lecithins from natural/vegetative (e.g., egg or soy lecithin) and synthetic origins are 10 known. The primarily type of lecithin is phosphatidylcholine (PC). Other types of lecithins include phosphatidylglycerol; phosphatidylinositol; sphingomyelin; and phosphatidylethanolamine. Derivatives of lecithin with saturated and unsaturated fatty acid side chains on PC, are also known, including: distearoylphosphatidyl choline; dipalmitoylphosphatidyl choline; and 15 dimyristoylphosphatidyl choline. As used herein, "lecithin" includes such derivatives of lecithin. Preferably, the lecithin ingredient comprises at least 75% PC.

Commercially available grades of soy lecithins include a fully 20 hydrogenated soy lecithin comprising 90% phosphatidylcholine available under the tradename Phospholipon 90H from American Lecithin Company and a soy lecithin comprising 75% phosphatidylcholine available under the tradename Lipoid-S75 from Vernon Walden, Inc. The amount of lecithin contained in the compositions of the present invention depends primarily on the 25 concentration of insoluble ingredients in the compositions. The amount of lecithin in the compositions of the present invention generally ranges from about 0.01 - 5%, preferably about 0.01 - 2% and most preferably is about 0.15%.

In addition to the water-insoluble drug compound and lecithin, the 30 compositions of the invention preferably contain a non-ionic surfactant. The most preferred nonionic surfactants are the surfactants known as polysorbates,

in particular polysorbates 20-80. Such polysorbate surfactants are commercially available under the trade name Tween from ICI Americas, Inc. Most preferred is polysorbate 20. The amount of surfactant contained in the compositions of the present invention generally ranges from about 0.01 - 2%,
5 preferably about 0.05 - 1%, and most preferably is about 0.1%.

In addition to the water-insoluble drug compound, lecithin and optional surfactant, the compositions, if intended for topical ophthalmic use, contain a tonicity-adjusting agent. The tonicity-adjusting agent is present in an amount
10 sufficient to cause the final composition to have an ophthalmically acceptable osmolality (generally about 150 - 450 mOsm, preferably 250 - 350 mOsm). If desired or required, the compositions of the present invention also contain one or more excipients. Conventional excipients include preservatives,
15 buffering agents, chelating agents or stabilizers, viscosity-enhancing agents and others. The chosen ingredients are mixed until homogeneous. After the solution is mixed, pH is adjusted (typically with NaOH or HCl) to be within a range suitable for the intended pharmaceutical use, generally within the range of pH 4.5 - 8.

Sodium chloride, mannitol, glycerin or the like may be used as the
20 isotonic agent; benzalkonium chloride, polyquaternium-1, benzyl alcohol or the like as the preservative; sodium hydrogenphosphate, sodium dihydrogenphosphate, boric acid or the like as the buffering agent; edetate disodium or the like as the chelating agent or chemical stabilizer; and sodium hydroxide, hydrochloric acid or the like as the pH controller.
25

The compositions of the present invention are preferably applied topically to the eye, ear or nose, but could be used elsewhere for topical or injected application.

30 The compositions of the present invention are prepared in a specific manner. It is essential that the water-insoluble drug compound is first mixed

with lecithin prior to being combined with the remainder of the composition. Preferably, the water-insoluble drug compound is mixed with both lecithin and a nonionic surfactant (preferably polysorbate 20 to 80) before being combined with the remainder of the composition. The presence of the surfactant provides a lower viscosity slurry than simply mixing hydrocortisone and lecithin alone. The lower viscosity achieved by the addition of the surfactant makes processing easier.

If not available as a "micronized" material, the water-insoluble drug compound can be sized in the presence of lecithin and optionally a surfactant. If the water-insoluble drug compound is sized prior to mixing with lecithin, then the mixing with lecithin step must occur prior to combining the water-insoluble drug compound with the remainder of the composition. Particle sizing techniques are known in the art and include ball milling, homogenization and micronization. As used herein, "mixing" includes simple mixing as well as sizing procedures.

The lecithin ingredient should be dispersed in water at a temperature above the phase transition temperature for the chosen grade of lecithin. In the case of phospholipon 90H, the phase transition temperature is approximately 51 °C. Therefore, Phospholipon 90H is preferably dispersed at a temperature of approximately 65 – 70 °C. A surfactant, if present, can be dispersed simultaneously with lecithin or added before or after lecithin is fully dispersed. After the surfactant and lecithin are dispersed, the water-insoluble drug compound (preferably micronized) is then dispersed to form a water-insoluble drug compound slurry. The water-insoluble drug compound is preferably added after removing the lecithin dispersion from heat, but before the lecithin dispersion cools to room temperature. The water-insoluble drug compound should be mixed with the lecithin dispersion for approximately 6 to 18 hours or more, preferably 12 hours, before being added to the remainder of the composition.

In a separate vessel, the remainder of excipients are dissolved in water to form an Excipient Solution. Although it is possible to add all of remainder of excipients simultaneously, provided that the vessel contains a sufficient amount of water, sequentially mixing and dispersing/dissolving, with each ingredient being dispersed or dissolved prior to the addition of the next, is preferred. For example, a buffering agent is added to purified water, then a preservative, and finally a tonicity-adjusting agent.

After the Excipient Solution has been prepared, it is combined with the water-insoluble drug compound slurry, then the pH is adjusted with an NaOH or HCl and the batch volume is adjusted with purified water.

The compositions described above are preferably prepared as follows.

15. 1. Add approx. 5 - 50% of the total batch volume of purified water to a compounding vessel and heat to a temperature above the transition temperature of the chosen grade of lecithin (in the case of Phospholipon 90H the preferred temperature is approximately 65 – 70 °C).
20. 2. Using a magnetic stir bar, disperse 50% of the total required amount of lecithin (preferably, Phospholipon 90H) and 50% of the total required amount of surfactant (preferably polysorbate 20) into the heated water of Step 1 until uniformly dispersed (generally about 10 – 20 min.). Remove from heat.
25. 3. Add the water-insoluble drug compound (preferably micronized) before the dispersion of Step 2 cools to room temperature and mix for approximately 12 hrs. (i.e., overnight).
30. 4. Prepare a solution by adding the following components in order and mix well allowing each to disperse or dissolve before adding the next: the remaining 50% of the total amount of lecithin (at elevated temperature), the remaining 50% of the total amount of surfactant, the preservative, the buffer (e.g., glacial acetic acid then sodium acetate (trihydrate)), and the tonicity-adjusting agent.

5. Add the water-insoluble drug dispersion of Step 3 to the solution of Step 4 (while mixing).
6. QS to 90% with purified water.
7. Measure and adjust pH to target pH with 1N NaOH and/or 1N HCl, then 5 QS to 100% with purified water.

The following examples are presented to illustrate further various aspects of the present invention, but are not intended to limit the scope of the invention in any respect.

10

Examples:

The formulations shown in Tables 1 and 2 were prepared (ingredient amounts shown as % w/w).

15

The physical stability of suspension formulations is commonly measured in two ways: resuspendability is assessed by measuring the number of inversions (also called strokes) required to redisperse sedimentation which forms after a sample stands undisturbed for a period of time; and rate of settling is assessed by observing the height in millimeters of the column of 20 sedimentation visible in a sample contained in a cylinder after shaking and then standing for a period of time. In order to record the rate of settling results, the following codes are used (in order of increasing turbidity): C: Clear Supematant Phase, LM: Light Milky Phase (less dense than Homogeneous phase), H: Homogenous Phase (initial homogeneous phase), D: Dense Phase (more 25 dense than Homogeneous Phase), S: Sediment. Larger sedimentation heights indicate less separation with less supematant liquid and less compaction of sedimentation. The physical stability of Formulations 1 – 10 was evaluated according to the methods described above and the results are shown in Tables 3 and 4.

Table 1.

| Ingredient | FORMULATION # | | | | |
|--|---------------|--------------|--------------|--------------|--------------|
| | 1 | 2 | 3 | 4 | 5 |
| Dexamethasone (micronized) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Hydroxyethyl Cellulose (NATROSOL 250HR) | — | — | — | 0.3 | 0.05 |
| Benzyl Alcohol | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Sodium Chloride | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Sodium Acetate (trihydrate) | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| Glacial Acetic Acid | 0.255 | 0.255 | 0.255 | 0.255 | 0.255 |
| Lecithin (Phospholipon 90H) | 0.15 | 0.15 | — | — | — |
| Polysorbate 20 (TWEEN 20) | 0.1 | — | 0.1 | 0.1 | 0.1 |
| Sodium Hydroxide | QS to pH 4.7 | QS to pH 4.7 | QS to pH 4.7 | QS to pH 4.7 | QS to pH 4.7 |
| Hydrochloric Acid | QS to 100 | QS to 100 | QS to 100 | QS to 100 | QS to 100 |
| Purified water | | | | | |

Table 2.

| Ingredient | Formulation # | | | | 10 |
|---|------------------|------------------|------------------|------------------|------------------|
| | 6 | 7 | 8 | 9 | |
| Dexamethasone Bell Oxil | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Hydroxyethyl Cellulose (NATROSOL 250-HR) | — | — | — | 0.3 | 0.05 |
| Benzyl Alcohol | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Sodium Chloride | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Sodium Acetate (trihydrate) | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| Glacial Acetic Acid | 0.255 | 0.255 | 0.255 | 0.255 | 0.255 |
| Lecithin (Phospholipon 90H) | 0.15 | 0.15 | — | — | — |
| Polyisobutene 20 (TWEEN 20) | 0.1 | — | 0.1 | 0.1 | 0.1 |
| Sodium Hydroxide | pH Adjust to 4.7 |
| Hydrochloric Acid | QS to 100 |
| Purified Water | | | | | |

Table 3. Resuspendability

| Resuspendability | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|
| Real Time | 1 | 2 | 1 | 60 | 3 | 1 | 2 | 5 | 35 | 3 |
| # Inversions after 4 days standing | | | | | | | | | | |
| Accelerated | | | | | | | | | | |
| 30 min. @ 500 rpm | 2,2 | 2,3 | 1,1 | 28,29 | 2,3 | 2,2 | 4,4 | 3,4 | 33,30 | 3,2 |
| # Inversions | <1,<1 | <1,<1 | <1,<1 | 3,4 | <1,<1 | <1,<1 | <1,<1 | <1,<1 | 2,2 | <1,<1 |
| Wrist shaking (sec.) | | | | | | | | | | |

Table 4. Rate of Settling

| Time | FORMULATION # | | | 6 | |
|---------|--|---|---|---|--|
| | 1 | 2 | 3 | | |
| Initial | 0-10 mi: H 0-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-10 mi: H 0-9.5 mi: LM 9.5-10 mi: C (flocculated susp. no sediment) | 0-10 mi: H 0-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-10 mi: H 0-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-10 mi: H 0-9.5 mi: LM 9.5-10 mi: C (no sediment) |
| 5 min | 0-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 9.5-10 mi: C (no sediment) |
| 10 min | 0-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 8.5-10 mi: C (no sediment) | 0-9.5 mi: S 0-2-5 mi: LM 9.5-10 mi: C (no sediment) |
| 15 min | 0-9.5 mi: S 0-5-9 mi: LM 9.5-10 mi: C (no sediment) | 0-9.5 mi: D 8-9 mi: LM 9-10 mi: C (flocculated susp. no sediment) | 0-9.5 mi: S 0-3-7 mi: LM (very few particles) 7-5-10 mi: C | 0-9.5 mi: S 0-3-7 mi: LM (very few particles) 9-10 mi: C | 0-9.5 mi: S 0-3-7 mi: LM (few particles) 9-10 mi: C |
| 20 min | 0-9.5 mi: S 0-5-9 mi: LM 9.5-10 mi: C (no sediment) | 0-8 mi: D 8-9 mi: LM 9-10 mi: C (flocculated susp. no sediment) | 0-9.5 mi: S 0-3-7 mi: LM (very few particles) 7-10 mi: C | 0-9.5 mi: S 0-3-7 mi: LM (few particles) 9-10 mi: C | 0-9.5 mi: S 0-3-7 mi: LM (few particles) 9-10 mi: C |
| 30 min | 0-9.5 mi: S 0-1-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-3 mi: S 3-9 mi: LM 9-10 mi: C (flocculated sediment) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (very few particles) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (very few particles) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (few particles) |
| 45 min | 0-9.5 mi: S 0-1-9.5 mi: LM 9.5-10 mi: C (no sediment) | 0-2.3 mi: S 2-3.9 mi: LM 9-10 mi: C (no particles) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (very few particles) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (very few particles) | 0-3 mi: S 0-3-4 mi: LM 4-10 mi: C (few particles) |
| 1 hr | 0-9.5 mi: S 0-1-9.5 mi: LM 9.5-10 mi: C (flocculated) | 0-2 mi: S 2-9 mi: LM 9-10 mi: C (very few particles) | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C |
| 2 hrs | 0-9.5 mi: S 0-1-9.5 mi: LM 9.5-10 mi: C (flocculated) | 0-1.5 mi: S 1-5-10 mi: C 1-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C |
| 3 hrs | 0-9.5 mi: S 0-3-9 mi: LM 9-10 mi: C (flocculated) | 0-1.2 mi: S 1-2-10 mi: C 1-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C | 0-3 mi: S 0-3-10 mi: C 0-10 mi: C |
| 1 Day | 0-9.5 mi: S 3-6-10 mi: C (flocculated) | 0-1 mi: S 1-10 mi: C | 0-0.2 mi: S 0-2-10 mi: C 0-10 mi: C | 0-0.1 mi: S 0-1-10 mi: C (with some haziness present) | 0-0.1 mi: S 0-1-10 mi: C (with some haziness present) |
| 3 Days | 0-2-2 mi: S 2-2-10 mi: C | 0-1 mi: S 1-10 mi: C | 0-0.2 mi: S 0-2-10 mi: C 0-10 mi: C | 0-0.2 mi: S 0-2-10 mi: C 0-10 mi: C | 0-0.2 mi: S 0-2-10 mi: C 0-10 mi: C |

Table 4. (cont'd)

| Time | FORMULATION # | | | | | 10 |
|---------|---|---|---|--|---|---|
| | 6 | 7 | 8 | 9 | 10 | |
| Initial | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) |
| 5 min | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) |
| 10 min | 0-10 mi: H (No Sediment) | 0-10 mi: D (Flocculated Suspension) | 0-10 mi: D (Flocculated Suspension) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) | 0-10 mi: H (No Sediment) |
| 15 min | 0-10 mi: H (No Sediment) | 0-1 mi: S (Flocculated Sediment) | 0-1 mi: S (Flocculated Sediment) | 0-0.6 mi: LM 9.5-10 mi: C (light sediment on bottom) | 0-10 mi: H (No Sediment) | 0-10 mi: LM (light sediment on bottom) |
| 20 min | 0-10 mi: H (No Sediment) | 0-1 mi: S (Flocculated Sediment) | 0-1 mi: S (Flocculated Sediment) | 0-0.5 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1.9 mi: D | 0-10 mi: H (No Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C |
| 30 min | 0-10 mi: H (No Sediment) | 0-1.9 mi: S (Flocculated Sediment) | 0-1.9 mi: S (Flocculated Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1.9-7.0 mi: D 7-10 mi: LM | 0-10 mi: H (No Sediment) | 0-0.08 mi: S 0.08-0.5 mi: LM 9.5-10 mi: C |
| 45 min | 0-10 mi: H (No Sediment) | 0-1.9 mi: S (Flocculated Sediment) | 0-1.9 mi: S (Flocculated Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1.9-10 mi: C | 0-10 mi: H (No Sediment) | 0-0.08 mi: S 0.08-0.5 mi: LM 9.5-10 mi: C |
| 1 Hr | 0-10 mi: H (No Sediment) | 0-1.7 mi: S (Flocculated Sediment) | 0-1.7 mi: S (Flocculated Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1.3 mi: S (Flocculated Suspension) | 0-10 mi: H (No Sediment) | 0-0.08 mi: S 0.08-0.5 mi: LM 9.5-10 mi: C |
| 2 Hrs | 0.9-7 mi: H 9.7-10 mi: C (Flocculated Suspension) | 0-1.3 mi: S (Flocculated Suspension) | 0-1.3 mi: S (Flocculated Suspension) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1.3-10 mi: C 0.1 mi: S (Flocculated Sediment) | 0-9.7 mi: H 9.7-10 mi: C 1-10 mi: C (Flocculated Suspension) | 0-0.08 mi: S 0.08-0.5 mi: LM 9.5-10 mi: C 1-10 mi: C |
| 3 Hrs | 0.9 mi: H 8-10 mi: C (Flocculated Suspension) | 0-1 mi: S (Flocculated Sediment) | 0-1 mi: S (Flocculated Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 1-10 mi: C 0.1 mi: S (Flocculated Sediment) | 0-9.7 mi: H 9.7-10 mi: C 1-10 mi: C 0-1 mi: S (Flocculated Suspension) | 0-0.1 mi: S 0.1-10 mi: C 0-1 mi: S 0.1-10 mi: C |
| 1 Day | 0-3.5 mi: S (Flocculated Sediment) | 0-0.8 mi: S (Flocculated Sediment) | 0-0.8 mi: S (Flocculated Sediment) | 0-0.1 mi: S 0.1-10 mi: C 0-0.7 mi: S (Flocculated Sediment) | 0-0.05 mi: S 0.05-0.5 mi: LM 9.5-10 mi: C 0-1 mi: S 0.1-10 mi: C 0-0.7 mi: C | 0-0.1 mi: S 0.1-10 mi: C 0-1 mi: S 0.1-10 mi: C |
| 3 Days | 0-2.1 mi: S (Flocculated Sediment) | 0-0.7 mi: S (Flocculated Sediment) | 0-0.7 mi: S (Flocculated Sediment) | 0-0.1 mi: S 0.1-10 mi: C 0-0.7 mi: C | 0-0.1 mi: S 0.1-10 mi: C 0-0.7 mi: C | 0-0.1 mi: S 0.1-10 mi: C 0-0.7 mi: C |

The results shown in Tables 3 and 4 demonstrate that the compositions of the present invention (Formulation #'s 1, 2, 6 and 7) have equivalent or superior physical stability to compositions containing a conventional polymeric suspending agent (Formulation #'s 4, 5, 9 and 10). When compared to Formulation #'s 5 and 10 (containing a relatively low concentration of a polymeric suspending agent such that after settling, the formulations would be relatively easy to resuspend), the formulations of the present invention have approximately equivalent resuspendability results but superior rate of settling results. See, for example, the data shown after 2 hours of settling. When compared to Formulation #'s 4 and 9 (containing a relatively high concentration of a polymeric suspending agent such that the rate of settling would be relatively low), the formulations of the present invention have approximately equivalent or superior rate of settling results but superior resuspendability results (2 – 4 inversions for Formulation #'s 1, 2, 6 and 7, but 28 – 33 inversions for Formulation #'s 4 and 9). See, for example, the data shown after 1 day of settling (where the greater the height of the "Sediment" phase, the more flocculated and easier to resuspend the formulation). Comparing the formulations of the present invention to Formulation #'s 3 and 8 (containing a surfactant but no lecithin or polymeric suspending agent), the resuspendability results were approximately equivalent, but the rate of settling results of the formulations of the present invention were superior. See, for example, the data shown after 1 day of settling.

The invention has been described by reference to certain preferred embodiments; however, it should be understood that it may be embodied in other specific forms or variations thereof without departing from its spirit or essential characteristics. The embodiments described above are therefore considered to be illustrative in all respects and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description.

WE CLAIM:

1. An aqueous pharmaceutical suspension composition comprising one or more water-insoluble drug compounds and a physical-stability enhancing additive consisting essentially of lecithin.
5
2. The composition of Claim 1 wherein the water-insoluble drug compound is present in an amount from about 0.001 – 5%.
10
3. The composition of Claim 1 wherein the water-insoluble drug compound is a steroid.
15
4. The composition of Claim 3 wherein the steroid is selected from the group consisting of dexamethasone; rimexolone; prednisolone; hydrocortisone; fluticasone propionate; budesonide; mometasone furoate monohydrate; and dexamethasone beloxil.
20
5. The composition of Claim 1 wherein the water-insoluble drug compound is selected from the group consisting of griseofulvin; carbamazepin; clofibrate; ketoprofen; 5-flurouracil; flurbiprofen; mefanamic acid; flufenamic acid; and crystalline beta escinic acid.
25
6. The composition of Claim 1 wherein the lecithin is present in an amount from about 0.01 - 5%.
7. The composition of Claim 6 wherein the lecithin is present in an amount from about 0.01 - 2%.
8. The composition of Claim 1 wherein the lecithin is selected from the group consisting of phosphatidylcholine; phosphatidylglycerol; phosphatidylinositol; sphingomyelin; phosphatidylethanolamine; distearoylphosphatidyl choline; dipalmitoylphosphatidyl choline; and dimirystoylphosphatidyl choline.
30

9. The composition of Claim 1 further comprising a surfactant.
10. The composition of Claim 9 wherein the surfactant is selected from the
5 group consisting of polysorbate 20 – 80 surfactants.
11. The composition of Claim 10 wherein the surfactant is present in an amount from about 0.01 – 2%.
- 10 12. The composition of Claim 9 further comprising one or more excipients selected from the group consisting of tonicity-adjusting agents; preservatives; buffering agents; chelating agents; anti-oxidants.
- 15 13. A method of preparing an aqueous pharmaceutical suspension composition comprising one or more water-insoluble drug compounds and a physical-stability enhancing additive consisting essentially of lecithin wherein the one or more water-insoluble drug compounds are mixed with lecithin and optionally a surfactant to form a water-insoluble drug compound slurry prior to being combined with any other excipients.
- 20 14. The method of Claim 13 wherein the one or more water-insoluble drug compounds are mixed with lecithin and a surfactant for about 6 to 18 hours prior to being combined with any other excipients.
- 25 15. The composition of Claim 10 wherein the water-insoluble drug compound is a steroid and is present in an amount from about 0.001 – 5%.
16. The method of Claim 13 wherein the lecithin is present in an amount from about 0.01 - 5%.
- 30 17. The method of Claim 16 wherein the lecithin is selected from the group consisting of phosphatidylcholine; phosphatidylglycerol; phosphatidylinositol;

sphingomyelin; phosphatidylethanolamine; distearoylphosphatidyl choline; dipalmitoylphosphatidyl choline; and dimyristoylphosphatidyl choline.

18. The method of Claim 13 wherein the surfactant is selected from the
5 group consisting of polysorbate 20 – 80 surfactants.

19. The method of Claim 18 wherein the surfactant is present in an amount
from about 0.01 – 2%.

10 20. The method of Claim 13 wherein the aqueous pharmaceutical
suspension composition comprises one or more excipients selected from the
group consisting of tonicity-adjusting agents; preservatives; buffering agents;
chelating agents; anti-oxidants.

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(54) Title: TOPICAL GEL DELIVERY SYSTEM

(57) **Abstract:** A composition is provided that has a viscosity of *lcsc* than about 15,000 cP and a pH of about 3.0 to 9.0 for treating a skin disorder in a human subject. The composition consists essentially of (a) a therapeutically-effective amount of at least one compound useful for treating such disorder, (b) a pharmaceutically-acceptable, lightly cross-linked polyacrylic acid polymer compatible with the compound, (c) optionally a water miscible solvent, (d) optionally a preservative, (e) optionally an oil phase component and suitable surfactant, and (f) water. The composition is useful for treating an inflammatory skin disorder, acne, or rosacea. The low viscosity composition has an advantage of being administered more accurately when combined with a container that administers the composition as drops.

TOPICAL GEL DELIVERY SYSTEM

BACKGROUND OF THE INVENTION

5 FIELD OF INVENTION

This invention relates to a composition for treating a skin disorder in a human, and a method of administering and preparing such composition.

BACKGROUND

- Skin disorders are a common problem in childhood, adolescence and adulthood.
10 Skin disorders can include, for example, acne, inflammatory diseases such as atopic eczema, or rosacea.

Acne vulgaris is a follicular disease characterized by pilosebaceous inflammations such as comedones, papules, pustules, cysts and nodules. Chiefly a disease of adolescence (and often a cause of emotional distress), acne originates endogenously and stems for 15 multifactorial influences. Major progressive factors in the development acne include hyperkeratosis of the follicular epithelium, increased sebum production, and proliferation of *Propionibacterium acnes*. These factors are primarily responsible for hyperkeratosis of the follicle lining, including retention of keratin and sebum, as well as the free fatty acid by-products of *P.acnes* metabolism which can lead to inflamed acne papules and 20 pustules.

Although acne may also be influenced by exogenous and hormonal factors, research has been centered around eliminating *P.acnes*, the most common cause of inflammation. To date, the pathogenesis of acne is not fully understood, and there is currently no cure for the disease. Many systemic and topical medications, such as 25 tetracycline, have been used to manage and control acne. None, however, is universally successful.

Acne treatment is typified by "polypharmacy", whereby physicians employ simultaneous treatment with a variety of modalities. The search for improved acne treatments has been widespread and continuous during the past several decades.
30 Enhanced cosmetic properties to encourage user compliance, the use of topical therapies in place of systemic drugs to reduce toxicity and side effects, and the introduction of new drugs and formulations represent the forefront of acne treatment advances.

The first use of a topical antibiotic, erythromycin, for the treatment of acne was reported by Fulton (Fulton, J.E. Jr. and Pablo, G. *Topical antibacterial therapy for acne*.

Study of the family of erythromycins. Arch. Dermatol. 110:83-86, 1974). Topical administration of these potent agents has the advantage of reduced side effects, particularly those caused by systemic drug effects, e.g., nausea, gastrointestinal upset, diarrhea, and vaginal yeast overgrowth.

5 Lincomycin antibiotics have been employed in the topical treatment of acne (U.S. Patent No. 3,969,516). Lincomycin was commercialized by Upjohn Co. (now Pharmacia & Upjohn) as Cleocin T Solution, Gel, Lotion and Pledgets. Cleocin T Gel was an improvement over Cleocin T Solution because of the elimination of alcohol and the ease of application to the facial skin for the treatment of acne. This gel is based on carbomer 10 934, NF.

Atopic dermatitis is a polygenic disease with an inherited predelection and strongly influenced by environmental factors. The condition affects infants, children, adolescents and adults and is allergic in nature. The distribution is symmetrical, typically involving the face, neck and flexural areas. Atopic dermatitis is chronic, relapsing and usually 15 pruritic. Topical treatment frequently includes topical corticosteroids, such as desonide, hydrocortisone valerate, fluocinolone acetonide, triamcinolone acetonide, betamethasone valerate, hydrocortisone butyrate, halobetasol propionate, betamethasone dipropionate, clobetasol propionate, difloransone diacetate, fluticasone propionate, budesonide or the like.

20 Rosacea is a chronic inflammatory eruption of the nose, face and other flushing areas of the skin. The disease is most common in middle aged women and is characterized by erythema, papules, pustules, telangiectasia and enlarged sebaceous glands. The cause etiology is not totally clear; however vasomotor lability and menopause are predisposing factors. The organism Demodex folliculorum is found frequently in the 25 contents of inflamed pustular follicles, and has a possible role in this skin disorder. Treatments include topical metronidazole and oral tetracycline type antibiotics.

SUMMARY OF THE INVENTION

One aspect of this invention is a composition having a pH of about 3.0 to about 9.0 and a viscosity of less than about 15,000 centipoise (cP) for treating a skin disorder in a 30 human subject. The composition comprises (a) a therapeutically-effective amount of at least one compound useful for treating such disorder, (b) a pharmaceutically-acceptable polyacrylic acid polymer compatible with the compound, (c) optionally a water miscible solvent, (d) optionally a preservative, (e) optionally an oil phase and surfactant, and (f) water.

Another aspect of the invention is a composition described above in combination with a container that accurately administers a portion of the composition for topical administration to a patient.

- Another aspect of the invention is a composition described above in combination
5 with labeling instructions for use in treating the skin disorder.

Still another aspect of the invention is a method for treating a skin disorder in a human subject, which method comprises administering a composition described above to an affected area of the subject's skin having such disorder in an amount and for a period of time sufficient to improve the skin disorder.

- 10 Still another aspect of the invention is a method for preparing a composition of this invention by combining water with a therapeutically-effective amount of a suitable compound and the polymer and optionally a water-miscible solvent and preservative. If a lotion is desired an oil phase is formed for integration with the aqueous phase.

- Other aspects of the invention may be apparent upon further reading the
15 specification and claims of the patent application.

SPECIFIC DESCRIPTION

- This invention provides a novel topical gel or lotion delivery system for the treatment of skin diseases, particularly acne vulgaris. One unique aspect of the system is the use of a polymeric material that provides a gel material that has a very low viscosity
20 but which is cosmetically elegant and aids in the administration process by providing a pourable composition that flows through a dropper tip easily.

The Composition

- One aspect of this invention is a composition having a pH of about 3 to about 9 and a viscosity of less than about 15,000 cP for treating a skin disorder in a human subject.
25 The composition comprises a therapeutically-effective amount of at least one compound useful for treating such disorder, a pharmaceutically-acceptable, lightly cross-linked polyacrylic acid polymer compatible with the therapeutically-effective compound, optionally a water miscible solvent, optionally a preservative, and water. The composition may include a solution of the active compound or a suspension. A lotion will also include
30 a pharmaceutically-acceptable oil phase emulsified with one or more surfactants.

The composition is useful to treat skin disorders, e.g. acne, rosacea, or inflammatory skin diseases such as atopic dermatitis. The composition will include an active agent that will be one compound alone or two or more compounds in combination.

The active agent can be an antibiotic, a corticosteroid, a retinoid, an anti-inflammatory imidazole, a non-steroidal anti-inflammatory agent (NSAID), or a combination.

An antibiotic is generally viewed as a drug that inhibits the growth of an unwanted microorganism. Representative examples of topical antibiotics include lincomycins, (e.g. 5 clindamycin), erythromycin, minocycline, and tetracycline, and the pharmaceutically-acceptable salts, esters, or prodrugs thereof. Preferred is clindamycin phosphate.

A "retinoid" is a keratolytic drug related to retinoic acid and generally includes chemical entities such as retinol and its esters and closely related naturally-occurring derivatives and structurally-related synthetic analogs. This includes, for example, retinol, 10 retinal, tretinoin (all-trans retinoic acid), isotretinoin, adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid), and the like. Of these, tretinoin is preferred.

Generally, a topical corticosteroid is a compound that is a structural modification of hydrocortisone (also known as cortisol) and that shows topical anti-inflammatory activity. Representative examples include those set forth in Table 65-1 at page 1575 of 15 "Goodman & Gilman's The Pharmacological Basis of Therapeutics," Eighth Edition, McGraw-Hill, Inc. (1993). Specific, non-limiting examples of topical corticosteroids include those mentioned in the "background" section of this application. Preferred corticosteroids, when used as a single active agent, include diflorasone diacetate, desonide, fluticasone propionate, halobetasol propionate, or budesonide. Halobetasol 20 propionate or desonide is most preferred when used as a single active ingredient.

Nonsteroidal anti-inflammatory agents (NSAIDs) are compounds that suppress the inflammatory response when topically applied by inhibiting prostaglandin synthesis or by other mechanisms of action. Examples may be found in Goodman and Gilman, *Ibid*. Representative examples include ibuprofen, indomethacin, diclofenac, and naproxen and 25 their salts. Preferred is diclofenac.

An anti-inflammatory imidazole is an imidazole compound that suppresses a topical inflammatory response. Metronidazole is a representative imidazole compound suitable for this invention.

In describing the details of the composition, the numerical ranges given herein are 30 those amounts that provide the functional results in the composition. Thus, the ranges are generally introduced with the term "about" to indicate a certain flexibility in the range, *i.e.* ±10% or less at the lower and upper numerical ranges given.

As mentioned, the active agent may be present alone or in combination. For example, a topical antibiotic, such as clindamycin phosphate, may be combined with a

topical corticosteroid. Where a formulation is designed primarily for application to the facial area, to treat acne for example, it is preferred to combine an antibiotic (e.g. clindamycin phosphate) with a less potent corticosteroid, such as desonide, hydrocortisone valerate, fluocinolone acetonide, hydrocortisone butyrate, or triamcinolone acetonide. The 5 topical antibiotic can also be combined with a retinoid, e.g. clindamycin phosphate and tretinoin or adapalene.

The composition of the invention will include a polymeric material that will be present in an amount sufficient to bring the viscosity of the composition to a level of not more than about 15,000 cP, preferably between about 100 and about 12,000, and more 10 preferably between about 300 and about 10,000. The viscosity is determined at room temperature (20-25°C) using a Brookfield viscometer model DV-I+, spindle #27 at 12 revolutions per minute (rpm). If the measured viscosity is less than 4,000 cP, spindle #21 should be used instead of #27. By keeping the viscosity below about 15,000 cP, the advantages of more appealing cosmetic characteristics and ease of accurate application 15 through improved flow and pourability are achieved.

The polymers that have been found to be particularly useful in the composition of the present invention are lightly cross-linked polyacrylic acid polymers which are available from B.F. Goodrich under the tradename CARBOPOL®. They are generically referred to as carbomers. The CARBOPOL polymers are hydrophilic polymers based on a 20 polyacrylic acid structure. For use in the present invention the lightly cross-linked polymers include CARBOPOL 910, 941, 971, and 981 and CARBOPOL ETD 2050.

Either CARBOPOL 941 or 981 is particularly valuable for the present invention because the viscosity of a gel based on CARBOPOL 941 or 981 is low relative to its concentration. This feature is the result of the low level of cross-linking within the 25 polymer structure in a neutralized aqueous system. In contrast polyacrylic acid polymers which display a high level of cross-linking, such as CARBOPOL 980 or 974P, produce gels with higher viscosity at comparable concentrations.

A 0.5% solution of either CARBOPOL 941 or 981 at pH 7.5 has a viscosity measurement of from 4,000 to 11,000 cP (Brookfield viscometer at 20 rpm) compared to a 30 viscosity measurement of from 40,000 to 60,000 cP for a comparable 0.5% solution of either CARBOPOL 940 or 980 (reference: B.F. Goodrich Product Guide, Bulletin 2).

This lower-level viscosity feature of the lightly cross-linked polyacrylic acid polymers, e.g. CARBOPOL 941 and 981, offers two advantages to the composition of the present invention. A gel made from one of these lightly cross-linked polymers provides

better skin feel and lubricity than a gel of comparable viscosity made from a highly cross-linked polymer. Second, a low viscosity gel can be administered very accurately by a dropper or drip-type dispenser as compared to other commercial products which are thicker gels that do not provide as accurate an application.

5 CARBOPOL 941 NF resin and its cosolvent polymerized alternative, CARBOPOL 981 NF resin, provide permanent emulsions and suspensions at low viscosities. The gels produced with these resins have excellent clarity. In ionic systems, they perform better than most of the other CARBOPOL resins and at concentrations below 1.5% in solvent systems. The polymers are available from B.F. Goodrich Specialty Chemicals, 9911

10 Brecksville Road, Cleveland, OH 44414-3247.

CARBOPOL resins are polymers of acrylic acid crosslinked with polyalkenyl ethers or divinyl glycol. The polymers are flocculated powders of primary particles averaging about 0.2 micron in diameter. The flocculated powders are agglomerates that average 2 to 7 microns as determined by Coulter Counter. These agglomerates cannot be broken down into the primary particle once produced.

15 Each primary particle can be viewed as a network structure of polymer chains interconnected by crosslinks. Without the crosslinks, the primary particle would be a collection of linear polymer chains intertwined but not chemically bonded. These linear polymers are soluble in a polar solvent, such as water. They swell in water up to 1000 times their original volume (and ten times their original diameter) to form a gel, especially when exposed to a pH environment above about 4-6. Since the pK_a of these polymers is 6.0±0.5, the carboxylate groups on the polymer backbone ionize, resulting in repulsion between the negative particles, which adds to the swelling of the polymer. Highly crosslinked polymers of this type do not dissolve in water, rather they form gels by forming homogeneous dispersions.

20 The glass transition temperature of CARBOPOL resin is 105°C (221° F) in powder form. However, the glass transition temperature drops dramatically as the resin comes into contact with water. The polymer chains start gyrating and the radius of gyration becomes bigger and bigger. Macroscopically, this phenomenon manifests itself as 25 swelling.

The aqueous composition of the invention, will optionally include a water miscible solvent and a preservative. The water miscible solvent (i.e. a cosolvent) will be present if needed, to assist in dissolving the active agent. The cosolvent may be a single component or a mixture. Examples include those that are miscible with water such as ethanol,

propylene glycol, glycerin, polyethylene glycol 400, and the like. Certain water-miscible solvents, such as glycerin or propylene glycol, also add beneficial humectant properties to the composition. Drug delivery and penetration into the skin can be modified by the water-miscible cosolvent composition.

- 5 The preservative useful in the composition is material that aids in ensuring a stable composition and/or prevents growth of bacteria. Thus, a preservative may be one or more of an antioxidant, a chelator, an antibacterial, or the like. Suitable preservatives include methylparaben, butylparaben, propylparaben, benzyl alcohol, sorbic acid, imidurea, thimerosal, propyl gallate, BHA, BHT, citric acid, disodium edetate, and the like. Another
10 optional additive is a fragrance. Generally, this will be present in a trace amount only and has no effect on the functioning of the composition.

A preferred composition, particularly for the treatment of acne, will exhibit a pH of about 3 to 9, preferably about 4 to 7, and most preferably at about 5 to 6. Thus, the composition may also include a pH-adjusting agent as needed at a level to adjust the pH to
15 the desired range. Such agents include many pharmaceutically-acceptable organic or inorganic bases, e.g., sodium hydroxide and tromethamine. The pH chosen will depend in part on the pH tolerance of the active agent chosen for the composition. The examples provide guidance for certain compounds and suitable pH values for the compositions.

Another aspect of this invention is an emollient embodiment, *i.e.*, a fluid emulsion
20 or lotion. This aspect of this invention is a composition having an internal oil phase dispersed with the aid of at least one surfactant, e.g. an emulsifier, in water. Suitable surfactants are well known in the art and include those referred to as anionic and nonionic agents. These are described in Remington: The Science and Practice of Pharmacy, Nineteenth Edition, Vol. 1 at p. 251. Representative surfactants include polysorbate 20,
25 polysorbate 40, polysorbate 60, polysorbate 80, sorbitan laurate, sorbitan oleate, sorbitan stearate, polyoxyethylene stearate, sodium lauryl sulfate, and lauryl sulfate. Oil phase components include those that are commonly used in the art such as mineral oil, petrolatum, stearyl alcohol, cetyl alcohol, isopropyl myristate, diisopropyl adipate, stearic acid, white wax, and the like.

30 The following Table sets forth operational and preferred ranges of the various components for a gel composition having an active ingredient, which may be a single compound or a combination of two or more compounds. The term surfactant means one or more surfactants, which includes wetting agents and emulsifiers.

TABLE A

| Component | % w/w | | |
|--------------------------|-------------|-----------|----------------|
| | Operational | Preferred | More Preferred |
| Active ingredient | 0.005-10.0 | 0.01-5.0 | 0.05-2.0 |
| Polyacrylic Polymer Acid | 0.05-3.0 | 0.05-1.0 | 0.1-0.5 |
| Cosolvent | 0.0-70.0 | 0.0-40.0 | 0.0-25.0 |
| Preservative | 0.0-3.0 | 0.01-1.0 | 0.05-0.25 |
| Surfactant* | 0.0-8.0 | 0.0-5.0 | 0.0-3.5 |
| Oil phase* | 0.0-50.0 | 0.0-25.0 | 0.0-15.0 |
| Water | QSAD 100 | QSAD 100 | QSAD 100 |
| Base | QS pH | QS pH | QS pH |

* Present for lotion

- The following Table B sets forth the operational, preferred, and more preferred concentrations of representative active ingredients that can beneficially be used in practicing our invention, whether alone or in combination. The exact amount will be readily determined by one of ordinary skill by referencing standard texts such as the Physicians Desk Reference or Goodman and Gilmann's referred to hereinbefore.

TABLE B

| Component | % w/w | | |
|----------------|-------------|-----------|----------------|
| | Operational | Preferred | More Preferred |
| Antibiotic | 0.1-5.0 | 0.5-2.0 | 0.5-1.0 |
| Corticosteroid | 0.005-2.5 | 0.01-1.0 | 0.05-0.1 |
| Retinoid | 0.005-0.5 | 0.05-0.1 | 0.025-0.05 |
| Imidazole | 0.1-5.0 | 0.5-2.0 | 0.75-1.0 |
| NSAID | 0.1-3.0 | 0.2-2.0 | 0.2-1.0 |

- To make an emulsion (*i.e.*, lotion) form of our invention as broadly set forth in Table A, the surfactant and oil phase component are included in the composition. The following table illustrates the manner in which the composition is modified to form a lotion.

TABLE C

| Component | % w/w | | |
|------------|-------------|-----------|----------------|
| | Operational | Preferred | More Preferred |
| Surfactant | 0.1-8.0 | 0.5-5.0 | 1.0-3.5 |
| Oil phase | 1.0-50.0 | 2.5-25.0 | 5.0-15.0 |

The preferred formula of the composition would either be preservative-free or have a decreased level of preservatives as compared to material that is commercially available. This is important because the presence of preservatives in a composition can result in irritation or allergic reaction of the skin. Reducing the possibility of skin irritation or allergic reaction in a composition provides a better product. Regarding compositions that contain clindamycin phosphate, the leading product is Cleocin T Gel. It is a clear viscous gel that tests have shown is not as well accepted as the less viscous material of the invention made with a more lightly cross-linked polymer. By controlling the viscosity of the gel at a low level it can be accurately dispensed from a clear plastic squeeze bottle rather than from an ointment tube. The advantage is two-fold. One is accurate dosage control by using a reduced orifice tip and improved product presentation for marketing. In addition, tests have shown that the less viscous material is cosmetically more elegant and will result more regular use.

In preparing a composition of this invention general formulation techniques known in the art of pharmaceutical science will be used. See, for example, Remington: The Science and Practice of Pharmacy, Nineteenth Edition, Mack Publishing Company (1995). Preparation of specific formulations may be found in the examples.

To prepare a gel with two active ingredients where one is suspended and the other is dissolved, first add the insoluble active to a water-miscible ingredient, or a portion of the water with a surfactant, to disperse. Separately, dissolve the other active and any other preservative ingredients in the purified water. Disperse the gelling agent in the aqueous solution with appropriate stirring. Then add the dispersion of the first active ingredient to the gel and mix well to blend. Last, add a pH adjusting agent to adjust the pH to the desired range. The preparation of a gel when both active ingredients are dissolved is similar, varying only in the first step. First, add the active with lower aqueous solubility to a solvent, blend of solvents, or water. Mix to dissolve. Separately, dissolve the other active and any preservative ingredients in the purified water. Disperse the gelling agent in the aqueous solution with appropriate stirring. Then add the solution of the first active to

the gel and mix well to blend. Last, add a neutralizing agent to adjust the pH to the desired range.

For a combination of an antibiotic e.g. clindamycin phosphate with a retinoid, such as tretinoin, three formulation approaches can be applied to a composition of the invention: 1) an aqueous gel, formed from a lightly crosslinked carbomer gelling agent, with the clindamycin phosphate dissolved and the tretinoin suspended; 2) an oil-in-water emulsion with the clindamycin phosphate dissolved in the water thickened with a lightly cross-linked carbomer gelling agent and the tretinoin dissolved in an internal liquid oil phase; and 3) a solution consisting of water and water-miscible organic solvents with the clindamycin phosphate and tretinoin both dissolved.

The following compositions are given as representative as the types of compositions useful in this invention.

Where the composition contains an antibiotic alone, for example clyndamycin phosphate, the composition has a pH of about 4 to 7 and contains

- 15 (a) about 0.5% to 2.0% w/w clindamycin phosphate,
- (b) about 0.1% to 0.4% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 15.0% to 25.0% w/w of a water miscible solvent,
- (e) less than about 0.2% w/w of a preservative, and
- 20 (g) QSAD purified water to 100% w/w.

Preferably such a composition has a pH of about 5 to 6 and contains

- (a) 1.0 to 1.5% w/w clindamycin phosphate,
- (b) 0.2% w/w of the polymer,
- (c) the base to adjust pH,
- 25 (d) 15.0% w/w propylene glycol and 5.0% w/w polyethylene glycol 400,
- (e) 0.1 – 0.15% w/w methylparaben, and
- (g) QSAD purified water to 100% w/w.

A gel composition where the antibiotic in clindamycin phosphate and the retinoid is tretinoin may contain

- 30 (a) (i) about 0.5% to about 2.0% w/w clyndamycin phosphate, and
(ii) about 0.01% to about 0.05% w/w tretinoin;
- (b) about 0.1% to about 0.5% w/w of the polymer;
- (c) the base to adjust pH;
- (d) about 10% to about 30% w/w of a water-miscible solvent;

- (e) less than about 0.2% of a preservative; and
- (g) QSAD purified water 100% w/w.

A lotion composition of clindamycin phosphate and tretinoin usefully will contain

- (a) (i) about 0.5% to about 2.0% w/w clindamycin phosphate and
 - 5 (ii) about 0.01% to about 0.05% w/w tretinoin;
- (b) about 0.1% to about 0.5% w/w of the polymer;
- (c) the base to adjust pH;
- (d) about 5% to about 30% w/w of a water-miscible solvent;
- (e) less than about 0.2% of a preservative;
- 10 (f) an oil phase in combination with at least one surfactant to form an emulsion; and
- (g) QSAD purified water 100% w/w.

Treatment

15 Another aspect of the invention is a method for treating a skin disorder in a human, which method comprises administering a composition to an affected area of the subject's skin having such disorder in an amount and for a period of time sufficient to improve the skin disorder, wherein the composition is described in this patent application. Preferably, the composition is administered once a day over the treatment period. Depending on the
20 patient's improvement, the treatment may extend for less than a week to two months or more. The progress of improvement may be monitored by the patient or by a physician.

The skin disorders which are treatable with the composition of the invention include acne vulgaris, rosacea, and various inflammatory conditions including atopic dermatitis. A discussion of these conditions may be found in the Merck Manual. For
25 example, acne vulgaris is an inflammatory disease affecting hair follicles and sebaceous glands. Lesions are most common on the face, but the neck, chest, upper back, and shoulders may also be affected.

The affected area of the subject's skin can be anywhere on the body in which the skin disorder exists. The amount of composition and period of administration time
30 sufficient to improve the skin disorder will be dependent on the subject and skin condition. Generally, a sufficient amount will be squeezed from a dropper tip of a squeeze bottle or an eye dropper onto the area affected and rubbed gently into the skin. Usually, no more than a few drops will be needed to apply to an affected area.

Article of Manufacture

Another aspect of the invention is an article of manufacture that comprises a composition for treating a skin disorder as described above in a suitable container, preferably in a dropper bottle, in combination with labeling instructions. The dropper
5 bottle can be made of any material, for example, glass, rigid plastic, or flexible plastic. Other means of administration are an eyedropper, or tube with a suitable small orifice size, such as an extended tip tube.

The composition of this invention may be, for example, filled and packaged into a plastic squeeze bottle (*i.e.*, 42 g). A suitable container-closure system for the package
10 presentation for the composition described in Table D.

TABLE D

| <u>NOMINAL SIZE</u> | <u>OVERFLOW CAPACITY</u> | <u>MATERIAL DESCRIPTION</u> | <u>MANUFACTURER</u> |
|---------------------|--------------------------|---|---|
| 1 oz | 46 cc | <ul style="list-style-type: none"> • Natural cylinder, round polypropylene bottle, 15/415 finish, Wheaton B-21411 • White low density polyethylene 15 mm dropper tip plug, Wheaton B-11048 • White polypropylene extended tip closure, Wheaton B-15044 | Wheaton Plastic Products 1101 Wheaton Avenue Milville, NJ 08332 |

The labeling instructions can come in the form of a pamphlet, a label applied to or associated with the packaging of the article of manufacture.

The labeling instructions provide for administering a composition of the invention
15 to an affected area of a subject's skin having a skin disorder, in an amount and for a period of time sufficient to improve the skin disorder. Printed labeling instructions are functionally related to the composition of the invention inasmuch as such labeling instructions describe a method to treat a skin disorder. The labeling instructions are an important aspect of the invention in that before a composition can be approved for any
20 particular use, it must be approved for marketing by the United States Food and Drug Administration. Part of that process includes providing a label that will accompany the pharmaceutical composition which is ultimately sold. While the label will include a definition of the composition and such other items such as the clinical pharmacology, mechanism of action, drug resistance, pharmacokinetics, absorption, bioavailability,
25 contraindications and the like, it will also provide the necessary dosage, administration and usage. Thus, the combination of the composition with the dropper bottle with

appropriate treatment instructions is important for the proper usage of the drug once it gets on the market. Such treatment instructions will describe the usage in accordance with the method of treatment set forth herein before.

Having now generally described this invention, the same will be better understood
5 by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any embodiment thereof, unless so specified.

In the following examples, the viscosity is determined at room temperature (20-
25°C) using a Brookfield viscometer model DV-I+, spindle #27 at 12 revolutions per
10 minute (rpm). If the measured viscosity is less than 4,000 cP, spindle #21 should be used instead of #27.

EXAMPLES**EXAMPLE I**

This example sets forth a pourable gel composition of this invention. The procedure set forth in steps a-f produces a composition according to Table I. The 5 composition is referred to as "Clindagel." An application to designate Clindagel as a trademark has been filed.

TABLE I

| COMPONENT | % W/W |
|---|------------------|
| Clindamycin phosphate, USP (equivalent to 1% clindamycin) | 1.19 |
| Methylparaben | 0.15 |
| CARBOPOL® 941 (or 981) | 0.20 |
| Propylene glycol | 15.0 |
| Polyethylene glycol 400 | 5.0 |
| Sodium hydroxide (10% solution) | QS pH 5.3 to 5.7 |
| Purified water | QSAD 100.00 |

The viscosity of this composition is about 1,000 cP.

- 10 a. Weigh approximately 90% of the purified water into a stainless steel kettle. Add the propylene glycol and polyethylene glycol 400. Stir with propeller mixer.
- b. At room temperature add methylparaben to step a) with continued stirring. Mix until dissolved.
- 15 c. While continuing to mix, add clindamycin phosphate to step b). Mix until dissolved.
- d. While continuing to mix, add CARBOPOL® 981 or 941 slowly to step c), avoiding clumping. Mix vigorously at room temperature until a uniform and lump-free dispersion is achieved.
- 20 e. While mixing, add sufficient sodium hydroxide, 10% solution, to achieve a pH of 5.3 to 5.7. Mix until uniform.
- f. Add the remaining water to make 100% and mix until uniform.

EXAMPLE II

This example shows the composition of a commercially available product containing clindamycin phosphate. The product is sold by Pharmacia as Cleocin T® Gel. The components and amounts were analyzed to be as follows:

5

TABLE II

| COMPONENT | % W/W |
|-------------------------|-----------|
| Clindamycin phosphate | 1.19 |
| Carbomer 934 P | 0.8 |
| Propylene glycol | 4.9 |
| Polyethylene glycol 400 | 10.2 |
| Sodium hydroxide | QS pH 5.4 |
| Methylparaben | 0.3 |
| Allantoin | 0.2 |
| Purified water | QSAD 100 |

The viscosity of this composition is about 20,000 cP.

EXAMPLE III10 **Comparison of Clindagel and Cleocin-T® Gel**

This example provides clinical data showing the advantages of a composition of the invention as compared to a known commercial composition.

A multi-center investigator-blind clinical trial was conducted comparing a composition of this invention (see Example I) Clindagel, once daily, and Cleocin-T® Gel (see Example II), twice daily (according to manufacturer's directions), in acne vulgaris. 15 Three hundred and twenty four patients, half in each group, were treated for up to 12 weeks. The investigator was "blinded" in that she/he did not know which treatment the patient used before the investigator evaluated the condition of the patient's acne.

Evaluations included inflammatory lesion count, total lesion count, physician's global assessment and skin-related side effects. Papules and pustules were considered inflammatory lesions. Total acne lesions included open and closed comedones in addition to inflammatory lesions. The physician's global severity assessment was based on a nine-point scale. At study end (12 weeks or last evaluation) it was concluded that Clindagel used once a day was equal in effectiveness to Cleocin-T® used twice daily and Clindagel had significantly fewer side effects. The data on lesion counts are summarized in Table III.

Table III: Improvement in Acne Lesions at Endpoint: ClindageITM Once Daily vs. Cleocin-T Gel Twice Daily.

| ACNE LESIONS | ClindageITM Once Daily | Cleocin-T® Gel Twice Daily | 95% Confidence Lower Bound |
|--------------|--|-------------------------------|-------------------------------|
| | Percent Change from Baseline (standard deviation) | | |
| Inflammatory | -50.90 (2.62) | -50.02 (2.62) | 0.897 |
| Total | -37.27 (2.44) | -39.52 (2.44) | 0.801 |

The physician's global assessment is summarized in Table IV.

Table IV: Summary of Number of Patients with a Two-Category Improvement from Baseline in the 9-Point Physician's Global Severity Assessment at Endpoint

| PHYSICIAN'S GLOBAL Assessment | ClindageITM Once Daily | Cleocin-T® Gel Twice Daily | 95% Confidence Lower Bound |
|----------------------------------|---------------------------|-------------------------------|-------------------------------|
| | Number of Patients | | |
| Improved by 2 categories | 84 | 84 | |
| Same or Worsened | 72 | 73 | 0.833 |
| TOTAL | 156 | 157 | |

The frequency of dermal side effects from Clindagel™ once daily and from Cleocin-T® twice daily are tabulated in Table V.

Table V: Summary Results of Frequency of Adverse Events Comparing Clindagel™ Once Daily and from Cleocin-T® Twice Daily.

| Category | ADVERSE EVENTS | | Fisher's Exact Test |
|---|-----------------------|----------------------------|---------------------|
| | Clindagel™ Once Daily | Cleocin-T® Gel Twice Daily | |
| Number of patients in safety evaluation | 168 | 165 | |
| Number of patients with at least one skin/appendage disorder reported | 2 | 13 | 0.003 |
| Frequency of local adverse reactions | 1.2% | 7.9% | |

5

EXAMPLE IV

This example sets forth the results of a user preference test (with vehicles, not actives) comprising a composition of this invention is shown in Example I (with CARBOPOL® 981) with the commercially available composition of Example II, (with 10 Carbomer 934 P). Table VI sets forth the formulation compositions.

The study was conducted amongst a normal subject patient population of 10 in order to evaluate the functional and cosmetic attributes using a half-face, paired, and symmetrical design.

Table VI

| Component | % w/w | |
|-------------------------|--------------|---------------|
| Vehicle Formulae: | Clindagel | Cleocin-T Gel |
| Carbomer 934P | — | 0.8 |
| Carbomer 981 | 0.2 | — |
| Propylene glycol | 15.0 | 4.9 |
| Polyethylene glycol 400 | 5.0 | 10.2 |
| Sodium hydroxide | qs to pH 5.5 | qs to pH 5.4 |
| Methylparaben | 0.15 | 0.3 |
| Allantoin | — | 0.2 |
| Purified water | QSAD 100 | QSAD 100 |

5

Test articles (gel vehicles) were identified by blinded identification code, thereby preventing test subject from knowing the identity of the test articles being applied. Each test pair involved test articles L vs. R, which were used on the left and right sides of the face respectively. The test articles assigned to L and R codes were varied so that each test article was randomly evaluated on R and L test locations and by order of application.

10 The subjects were equally balanced for sex. The mean age of the population was 34 years old within an age range of 25-44 years.

The following attributes were assessed during and after application: spreadability, feel/texture during application, ease of application, ability to rub the gel into the skin, 15 drying time on the skin, skin feel after application, overall cosmetic preference, and usability of the product. Each gel was evaluated for its functional and cosmetic attributes on a scale of 1-6, with 1 being *Unacceptable* and 6 being *Excellent*.

Of the nine subjects with a preference for one of the test articles, 67% preferred Clindagel vehicle over Cleocin-T vehicle. The degree of preference of Clindagel over 20 Cleocin-T was judged "moderate" to "great" in 100% of those tested. The data are tabulated in Table VII.

Table VII. Vehicle Preference By Subject

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | TOTAL |
|-----------|----|----|----|----|----|----|----|----|----|----|-------|
| Age | 26 | 39 | 44 | 42 | 25 | 28 | 42 | 35 | 33 | 26 | |
| Sex | F | F | M | M | F | F | M | F | M | M | |
| Cleocin-T | | | | | | P | P | P | NP | 3 | |
| Clindagel | P | P | P | P | P | P | | | NP | 6 | |

P = Preferred

NP = No Preference

F = Female

M = Male

There was a significantly higher score for Clindagel vehicle than for Cleocin-T gel vehicle in four of the specific attributes, and no significant difference in two of those attributes (Table VIII). Clindagel vehicle scored marks of "Very Good" in three of the six attribute categories and marks of "Good" in three other. Cleocin-T gel vehicle scored marks of "Very Good" in one category, "Good" in four categories and "Fair" in one category.

Table VIII. Frequency of Higher Score of Clindagel Vehicle and Cleocin-T Gel Vehicle

| <i>Functional and Cosmetic Attribute:</i> | Frequency of Higher Score (%) | | No Preference |
|---|-------------------------------|-----------|---------------|
| | CLINDAGEL | CLEOCIN-T | |
| Spreadability | 30% | 30% | 40% |
| Feel/texture during application | 50% | 30% | 20% |
| Ease of application | 20% | 30% | 50% |
| Ability to rub gel into skin | 50% | 20% | 30% |
| Length of drying time | 70% | 20% | 10% |
| Skin feel after application | 50% | 30% | 20% |

Forty percent of test subjects commented independently that the Clindagel vehicle was "runny" or "watery" upon application. This was also reflected in the "Ease of Application" attribute, where Cleocin-T had a slightly higher score. 50% of test subjects commented independently on their face feeling "sticky" after application of the Cleocin-T vehicle. 80% of test subjects indicated that they would use the Clindagel vehicle as a facial medication product. Only 30% of those tested indicated that they would use Cleocin-T vehicle as a facial medication product.

EXAMPLE V**Stability Study of ClindageTM with Clindamycin Phosphate as Active Ingredient**

This example provides laboratory data showing stability of Clindagel (Example I) for at least 18 months at 25°C. Clindagel was tested for the stability of the active ingredient, clindamycin phosphate, over time at controlled room temperature (i.e., 25°C and 60% relative humidity). A stability-indicating, high performance liquid chromatography assay was used to assess remaining clindamycin phosphate potency, expressed as clynamycin, during the experiment. Based on the data shown in Table IX, Clindagel is projected to have a commercial shelf life of about 24 months.

The estimated shelf life was calculated from the 95% confidence interval around the least squares fit to the available data. The projected shelf life is the time at which the drug potency reaches 90% of label claim (as allowed by the USP). The software used for the statistical analysis is named "SLIMStat⁺" and is sold by Metrics, Inc., P.O. Box 4035, Greenville, NC 27836, phone 252-752-3800.

Table IX: Room Temperature Stability Assessment of Clindamycin Potency in ClindageTM, 1%.

| | Percent Clindamycin by Weight | | | | | |
|-----------------------------|-------------------------------|---------|----------|----------|-----------|-----------|
| | Initial | 1 month | 2 months | 6 months | 12 months | 18 months |
| Clindamycin Phosphate Assay | 1.028 | 1.017 | 1.009 | 1.004 | 0.983 | 0.959 |

EXAMPLE VI**Clindamycin Phosphate-Tretinoiin Combination Composition**

Section 1

This section of this example describes two gel compositions of the invention in which the active ingredients are clindamycin phosphate and tretinoiin.

Two pourable gel compositions containing a combination of clindamycin phosphate and tretinoiin were made according to the invention. In Formulation A, the gel had a pH of about 5.5 and a viscosity of about 6100 cP. In Formulation B, the gel exhibited a pH of about 4.7 and a viscosity of about 6,000 cP. See quantitative formulae in Table X. This example illustrates the utility of our invention in the preparation of physically and chemically stable gel formulations.

Table X: Quantitative compositions of two combination Clindamycin Phosphate/Tretinoin gel formulations:

| Component | A % w/w | B % w/w |
|-----------------------------|--------------|--------------|
| Tretinoin | 0.025 | 0.025 |
| Clindamycin Phosphate | 1.21 | 1.21 |
| Propyl Gallate | --- | 0.02 |
| BHA | 0.02 | --- |
| Citric Acid | --- | 0.05 |
| Disodium Edetate | 0.05 | 0.05 |
| Polysorbate 80 | 5.0 | 0.08 |
| Propylene Glycol | 5.0 | --- |
| PEG 400 | 20.0 | --- |
| Glycerin | --- | 10.0 |
| Methylparaben | 0.1 | 0.15 |
| CARBOPOL 981 | 0.5 | 0.5 |
| Tromethamine (10% in water) | QS to pH 5.5 | QS to pH 4.5 |
| Purified Water | QSAD 100 | QSAD 100 |

Method of preparation: Formula A

- 5 a. Combine the propylene glycol, polyethylene glycol 400, and polysorbate
 80. Add the tretinoin and stir to dissolve.
- b. In a separate container dissolve the disodium edetate, methylparaben, and
 butylated hydroxyanisole in the purified water.
- c. Add the clindamycin phosphate to the aqueous solution of step b and stir to
 10 dissolve.
- d. Disperse the CARBOPOL 981 into the aqueous solution with high-speed
 stirring.
- e. Add the tretinoin drug phase to the aqueous CARBOPOL dispersion with
 stirring and then add the tromethamine and mix to form a homogeneous gel.

15 Method of preparation of Formula B

- a. Combine the glycerin and polysorbate 80. Add the tretinoin and stir to wet
 and disperse.

- b. In a separate container dissolve the propyl gallate, citric acid, disodium edetate, methylparaben, and butylated hydroxyanisole in the purified water.
- c. Add the clindamycin phosphate to the aqueous solution of step b and stir to dissolve.
- 5 d. Disperse the CARBOPOL 981 into the aqueous solution with high-speed stirring.
- e. Add the tretinoin drug phase to the aqueous CARBOPOL dispersion with stirring and then add the tromethamine and mix to form a homogeneous gel.

Section 2

10 This Section of this example describes two additional compositions that are slight modifications of Formulas A and B, wherein the preservatives have been changed or adjusted. The formulas are given below. C is similar to A, and D is similar to B.

TABLE XI

| Component | C % w/w | D % w/w |
|-----------------------|--------------|--------------|
| Clindamycin Phosphate | 1.24 | 1.24 |
| Tretinoin | 0.025 | 0.025 |
| Propyl Gallate | — | 0.02 |
| BHA | 0.02 | — |
| Citric Acid | — | 0.05 |
| Disodium Edetate | 0.05 | 0.05 |
| Methylparaben | — | 0.15 |
| Propylparaben | — | 0.03 |
| Benzyl Alcohol | 1 | — |
| Polysorbate 80 | 5 | 0.08 |
| Propylene Glycol | 5 | — |
| PEG 400 | 20 | — |
| Glycerin | — | 10 |
| Tromethamine (10%) | qs to pH 5.5 | qs to pH 4.5 |
| CARBOPOL 981 | 0.5 | 0.5 |
| Purified Water | qsad 100 | qsad 100 |

15 In making formula C, the 0.1% methylparaben preservative in Formula A was replaced with 1.0% benzyl alcohol. In Formula D, 0.03% propylparaben was added as an additional preservative (because the combination of methylparaben and propylparaben is sometimes a better preservative system). Methods of preparation:

- Formula C is prepared similarly to Formula A, except that methylparaben would be omitted from step "b," and the benzyl alcohol would be added to step "a."
 - Formula D is prepared similarly to Formula B; propylparaben would be added to step "b."
- 5

The Formula C gel has a pH about 5.5 and a viscosity about 9000 cP. The Formula D gel has a pH about 4.6 and a viscosity about 4100 cP.

EXAMPLE VII

10 **Assessment of Chemical Stability of Tretinoin in Formulations A and B from Example VI.**

This example provides laboratory data showing the stability of tretinoin in two compositions of the invention under accelerated test conditions.

15 Tretinoin is known to be relatively unstable, therefore, the chemical stability of these combination formulations was assessed in a 12-week accelerated stability study. The gels were packaged in amber glass vials, 8 grams each, and stored at 40°C. High performance liquid chromatography assays were performed initially and at 2, 4, and 12 weeks using the method for tretinoin cream (USP 24, page 1684). Both compositions were found to retain their potency in this accelerated study. Table XII summarizes the 20 chemical stability results.

Table XII: Accelerated Temperature (40°C) Stability Assessment of Tretinoin Potency in Formulations A and B, Example VI.

| Tretinoin Concentration (% w/w) | TIME IN WEEKS | | | |
|------------------------------------|---------------|--------|--------|--------|
| | 0 | 2 | 4 | 12 |
| Formula A | 0.0210 | 0.0228 | 0.0236 | 0.0231 |
| Formula B | 0.0236 | 0.0231 | 0.0234 | 0.0234 |

EXAMPLE VIII**Composition of Combination Gel Formulation**

This example teaches how to modify a known commercial composition of Example II to include tretinoin.

5 A combination gel formulation of tretinoin 0.025%, and clindamycin 1% was made by spatulating tretinoin powder and propyl gallate (an antioxidant to retard oxidative loss of tretinoin) into Cleocin®T gel (Example II). The quantitative formula is shown in Table XIII.

TABLE XIII

| Component | Amount |
|----------------|-----------------|
| Tretinoin | 0.0074g |
| Propyl Gallate | 0.0145g |
| Cleocin®T gel | 28.0000g |
| TOTAL | 28.0219g |

10

The tretinoin and propyl gallate were accurately weighed, placed on a glass plate, and incorporated into the Cleocin®T gel with a spatula. During spatulation, the product was protected from light. The resulting product was a smooth, clear light yellow gel with a pH of 5.7 and a viscosity of about 20,000 cP.

15

EXAMPLE IX**Physical Stability Studies of the Compositions of Example VI (Formula A) and Example VIII**

This example compares a composition of the invention (Example VI, Formula A)
20 with a modified commercial composition (Example VIII) with regards to crystal growth.

The physical stability of Example VI, Formula A and Example VIII, was assessed over a 4-week period at 5°C, 40°C and 50°C. The stability evaluation was based on careful physical examination for description at initial, 2 week and 4 week times. At study end, microscopic examination was performed to check for precipitation of tretinoin and crystal growth. As illustrated in the data summary below (Table XIV), the modified commercial formulation, Cleocin®T gel, was physically unstable compared to a composition of the invention, Example VI (Formula A).

Table XIV

| Example VI (Formula A) | Description: Clear Light Yellow Gel | | |
|-----------------------------------|--|----------------|---------------------------------|
| | Initial | 2 weeks | 4 weeks |
| 5°C | Clear | Clear | Clear – no crystals |
| 40°C | Clear | Clear | Clear – no crystals |
| 50°C | Clear | Clear | Clear – no crystals |
| Example VIII | Initial | 2 weeks | 4 weeks |
| | Clear | Hazy | Hazy – Crystals to 1200 microns |
| | Clear | Translucent | Hazy – Crystals to 1200 microns |
| | Clear | Translucent | Hazy – Crystals to 1200 microns |

EXAMPLE X

This example sets forth a lotion composition of this invention comprising two active ingredients: an antibiotic, i.e., clindamycin phosphate, and a retinoid, i.e., tretinoin. The components for this lotion are set forth in Table XIV.

5

Table XIV

| Component | % w/w |
|-----------------------------|--------------|
| Clindamycin Phosphate | 1.21 |
| Tretinoin | 0.025 |
| Stearyl Alcohol | 5.00 |
| Diisopropyl Adipate | 6.00 |
| PEG 40 Stearate (Myrij 52) | 2.00 |
| Sorbitan Stearate (Span 60) | 2.00 |
| Butylated Hydroxytoluene | 0.02 |
| Propylene Glycol | 5.00 |
| Methylparaben | 0.15 |
| Propylparaben | 0.03 |
| Citric Acid | 0.05 |
| Disodium Edetate | 0.10 |
| CARBOPOL 981 | 0.10 |
| Tromethamine (10%) | qs pH 5.5 |
| Purified Water | qsad 100 |

The viscosity of this composition is about 7,000 cP.

Method of preparation:

- a. Combine the propylene glycol and purified water. Add the methylparaben, propylparaben, citric acid, and disodium edetate and stir to dissolve.
- 10 b. Add the clindamycin phosphate to step "a" and stir to dissolve.
- c. Add the Carbopol 981 to step "b" and stir to form a homogeneous dispersion.
- d. Warm step "c" water phase to between 60°C and 70°C.
- 15 e. Combine the stearyl alcohol, PEG 40 stearate, sorbitan stearate, and butylated hydroxytoluene and warm to melt at between 60°C to 70°C.
- f. Add the tretinoin to the diisopropyl adipate and stir to dissolve.
- g. With high-speed stirring add step "c" oil phase and step "f" drug phase sequentially to step "d" water phase and mix well.
- 20 h. Cool emulsion with continued stirring.

1. Add the tromethamine solution and stir to form a homogeneous emulsion.
Cool to room temperature with continued stirring.

EXAMPLE XI

5 This example sets forth a pourable gel composition of this invention which gel contains a corticosteroid. Such formulation is suitable for treating inflammatory skin conditions such as atopic dermatitis.

Table XV

| Component | % by weight |
|------------------------------------|-------------|
| Halobetasol propionate, micronized | 0.05 |
| Docusate sodium | 0.10 |
| CARBOPOL® 981 | 0.3 |
| Propylene glycol | 12 |
| Methylparaben | 0.1 |
| Propylparaben | 0.02 |
| Tromethamine | QS pH 6.5 |
| Purified water | QSAD 100.00 |

- 10 The viscosity of this composition is about 6200 cP.
- a. Dissolve the methylparaben and propylparaben in the propylene glycol at room temperature using a propeller mixer.
 - b. Weigh 70% of the formula weight of purified water and slowly add the solution from step "a" while mixing with propeller mixer.
 - c. While continuing to mix, add CARBOPOL® 981 slowly to step "b." Mix at room temperature until a smooth and uniform dispersion is produced.
 - d. To 10% of the formula weight of water add the docusate sodium and mix until fully dissolved. To facilitate dissolution the mixture may be warmed to 40-50°C, and then cooled to room temperature when dissolution is complete.
 - e. Disperse the micronized halobetasol propionate in step "d" with a propeller mixer or preferably a homogenizer of the rotor-stator type.
 - f. Add step "e" to step "c" using propeller mixer to uniformly disperse the drug material.
 - 25 g. Dissolve the tromethamine in 10 times its weight in purified water. While mixing, use the tromethamine solution to adjust the pH and thicken the gel. Continue incremental additions until a pH of about 6.5 is attained.

- h. Add water to make 100% of the batch size and mix until homogeneous with a propeller-type mixer.

EXAMPLE XII

5 This example sets forth yet another pourable gel composition of this invention. The formulation contains metronidazole for topical application to the skin areas affected, for example, with rosacea.

Table XVI

| Component | % by weight |
|----------------|-------------|
| Metronidazole | 0.75 |
| Methylparaben | 0.12 |
| Propylparaben | 0.03 |
| CARBOPOL® 981 | 0.25 |
| Glycerin | 5.00 |
| Trolamine | QS pH 8 |
| Purified Water | QSAD 100 |

- 10 The viscosity of this composition is about 4700 cP.
- a. Weigh 90% of the formula weight of purified water, metronidazole, glycerin, methyl-paraben and propylparaben into a suitable stainless steel container. Mix vigorously at room temperature until all components are dissolved. A propeller-type mixer is particularly suitable.
- 15 b. While continuing to mix, slowly add the CARBOPOL®. Mix until a lump-free dispersion is attained.
- c. Mix the trolamine with an equal part of purified water. Use this solution to adjust the pH to about 8 with incremental additions while mixing.
- d. Add the balance of the purified water to make 100% and mix until a homogeneous gel is produced.
- 20

EXAMPLE XIII

This example sets forth a pourable gel composition of this invention which gel contains a NSAID agent.

Table XVII

| Component | % by weight |
|--------------------------------|------------------|
| Naproxen | 1.00 |
| Octoxynol 9 | 0.10 |
| CARBOPOL® 981 | 0.30 |
| Propylene glycol | 5.00 |
| Glycerin | 5.00 |
| Benzyl alcohol | 1.00 |
| Sodium hydroxide, 10% solution | QS pH 3.0 to 3.5 |
| Purified water | QSAD 100.00 |

The viscosity of this composition is about 4200 cP.

- a. Mix the benzyl alcohol, glycerin and propylene glycol together at room temperature using a propeller mixer.
- 5 b. Weigh 70% of the formula weight of purified water and slowly add the solution from step "a" while mixing with propeller mixer.
- c. While continuing to mix, add CARBOPOL® 981 slowly to step "b." Mix at room temperature until a smooth and uniform dispersion is produced.
- 10 d. To 2-5% of the formula weight of water add the octoxynol 9 and mix until fully dissolved.
- e. Disperse the naproxen in step "d" with a propeller mixer or a homogenizer.
- f. Add step "e" to step "c" using propeller mixer to uniformly disperse the drug material.
- 15 g. While mixing, use the sodium hydroxide solution to adjust the pH. Continue incremental additions until a pH of 3.0 to 3.5 is attained.
- g. Add water to make 100% of the batch size and mix until homogeneous with a propeller-type mixer.

20 EXAMPLE XIV

This example sets forth a pourable gel composition of this invention. The procedure set forth in steps a-f produces a composition according to Table I. The composition is designed to be used on sensitive skin and contains clindamycin phosphate as the active ingredient.

Table XVIII

| COMPONENT | % W/W |
|---|------------------|
| Clindamycin phosphate, USP (equivalent to 1% clindamycin) | 1.19 |
| Methylparaben | 0.15 |
| CARBOPOL® 941 (or 981) | 0.20 |
| Polyethylene glycol 400 | 5.0 |
| Sodium hydroxide (10% solution) | QS pH 5.3 to 5.7 |
| Purified water | QSAD 100.00 |

The viscosity of this composition is about 1,000 cP.

- a. Weigh approximately 90% of the purified water into a stainless steel kettle.
5 Add the polyethylene glycol 400. Stir with propeller mixer.
- b. At room temperature add methylparaben to step a) with continued stirring.
Mix until dissolved.
- c. While continuing to mix, add clindamycin phosphate to step b). Mix until dissolved.
- 10 d. While continuing to mix, add CARBOPOL® 981 or 941 slowly to step c), avoiding clumping. Mix vigorously at room temperature until a uniform and lump-free dispersion is achieved.
- e. While mixing, add sufficient sodium hydroxide, 10% solution, to achieve a pH of 5.3 to 5.7. Mix until uniform.
- 15 f. Add the remaining water to make 100% and mix until uniform.

EXAMPLE XV

This example sets forth a pourable gel composition of this invention which gel contains a corticosteroid (desonide) as the sole active ingredient. Such formulation is
20 suitable for treating inflammatory skin conditions such as atopic dermatitis.

Table XIX

| Component | % by weight |
|----------------------|-------------|
| Desonide, micronized | 0.05 |
| Docusate sodium | 0.10 |
| CARBOPOL® 981 | 0.3 |
| Propylene glycol | 5.0 |
| Methylparaben | 0.2 |
| Propylparaben | 0.3 |
| Tromethamine | QS pH 5.5 |
| Purified water | QSAD 100.00 |

The viscosity of this composition is about 6200 cP.

- a. Dissolve the methylparaben and propylparaben in the propylene glycol at room temperature using a propeller mixer.
- 5 b. Weigh 70% of the formula weight of purified water and slowly add the solution from step "a" while mixing with propeller mixer.
- c. While continuing to mix, add CARBOPOL® 981 slowly to step "b." Mix at room temperature until a smooth and uniform dispersion is produced.
- 10 d. To 10% of the formula weight of water add the docusate sodium and mix until fully dissolved. To facilitate dissolution the mixture may be warmed to 40-50°C, and then cooled to room temperature when dissolution is complete.
- e. Disperse the micronized desonide in step "d" with a propeller mixer or preferably a homogenizer of the rotor-stator type.
- 15 f. Add step "e" to step "c" using propeller mixer to uniformly disperse the drug material.
- g. Dissolve the tromethamine in 10 times its weight in purified water. While mixing, use the tromethamine solution to adjust the pH and thicken the gel.
- 20 h. Continue incremental additions until a pH of about 5.5 is attained.
- i. Add water to make 100% of the batch size and mix until homogeneous with a propeller-type mixer.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

The subject matter claimed is:

1. A composition having a pH of about 3 to about 9 and a viscosity of less than about 15,000 cP for treating a skin disorder in a human subject, which composition consists essentially of

- 5 (a) a therapeutically-effective amount of at least one compound useful for treating such disorder,
- (b) a pharmaceutically-acceptable, lightly cross-linked polyacrylic acid polymer compatible with the compound,
- (c) a pharmaceutically-acceptable base to adjust pH,
- 10 (d) optionally a water miscible solvent,
- (e) optionally a preservative,
- (f) optionally at least one surfactant alone or in combination with an oil phase, and
- (g) water.

15 2. The composition of Claim 1, wherein the compound is an antibiotic, imidazole, retinoid, corticosteroid, or a non-steroidal anti-inflammatory drug (NSAID).

3. The composition of Claim 2, wherein the compound is an antibiotic alone or in combination with a corticosteroid or a retinoid.

4. The composition of Claim 3, wherein the compound is an antibiotic alone.

20 5. The composition of Claim 4, wherein the antibiotic is clindamycin phosphate.

6. The composition of Claim 5 having a pH of about 4.0 to 7.0, which composition consists essentially of

- 25 (a) about 0.5% to 2.0% w/w clindamycin phosphate,
- (b) about 0.1% to 0.4% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 15.0% to 25.0% w/w of a water miscible solvent,
- (e) less than about 0.2% w/w of a preservative, and
- (g) QSAD purified water to 100% w/w.

7. The composition of Claim 6, wherein the water miscible solvent of part (d) is present at an amount of about 3.0% to 10.0% w/w.

8. The composition of Claim 6 having a pH of about 5.0 to 6.0, which composition consists essentially of:

- 5 (a) about 1.0 to 1.5% w/w clindamycin phosphate,
- (b) about 0.2% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 5.0% w/w polyethylene glycol 400,
- (e) about 0.1 – 0.15% w/w methylparaben, and
- 10 (g) QSAD purified water to 100% w/w.

9. The composition of Claim 6 having a pH of about 5.0 to 6.0, which composition consists essentially of:

- (a) about 1.0 to 1.5% w/w clindamycin phosphate,
- (b) about 0.2% w/w of the polymer,
- 15 (c) the base to adjust pH,
- (d) about 5.0% w/w polyethylene glycol 400,
- (e) about 0.1 – 0.15% w/w methylparaben, and
- (g) QSAD purified water to 100% w/w.

10. The composition of Claim 3, wherein an antibiotic is combined with a corticosteroid, the antibiotic is clindamycin phosphate, and the corticosteroid is desonide, hydrocortisone valerate, fluocenolone acetinide, hydrocortisone butyrate, or triamcinolone acetonide.

20 11. The composition of Claim 3, wherein an antibiotic is combined with a retinoid, the antibiotic is clindamycin phosphate, and the retinoid is tretinoïn.

25 12. The composition of Claim 11 having a pH of about 4 to 7, which composition is a gel consisting essentially of

- (a) (i) about 0.5% to about 2.0% w/w clindamycin phosphate, and

- (ii) about 0.01% to about 0.05% w/w tretinoin;
 - (b) about 0.1% to about 0.5% w/w of the polymer;
 - (c) the base to adjust pH;
 - (d) about 10% to about 30% w/w of a water-miscible solvent;
 - 5 (e) less than about 0.2% of a preservative; and
 - (g) QSAD purified water 100% w/w.
13. The composition of Claim 11 having a pH of about 5 to 6, which composition is a lotion consisting essentially of
- (a) (i) about 0.5% to about 2.0% w/w clindamycin phosphate and
 - 10 (ii) about 0.01% to about 0.05% w/w tretinoin;
 - (b) about 0.1% to about 0.5% w/w of the polymer;
 - (c) the base to adjust pH;
 - (d) about 5% to about 30% w/w of a water-miscible solvent;
 - (e) less than about 0.2% of a preservative; and
 - 15 (f) an oil phase in combination with at least one surfactant to form an emulsion; and
 - (g) QSAD purified water 100% w/w.
14. The composition of Claim 1, wherein the compound is a non-steroidal anti-inflammatory drug.
- 20 15. The composition of Claim 14, wherein the compound is naproxen or diclofenac or a pharmaceutically-acceptable salt thereof.
16. The composition of Claim 2, having a corticosteroid as the sole active agent.
17. The composition of Claim 16, wherein the corticosteroid is diflorasone diacetate, desonide, fluticasone propionate, halobetasol propionate, or budesonide.
- 25 18. The composition of Claim 17, wherein the corticosteroid is halobetasol propionate.
19. The composition of Claim 17, wherein the corticosteroid is desonide.

20. The composition of Claim 19 having a pH of about 4.0 to 7.0, which composition consists essentially of

- (a) about 0.01% to 0.1% w/w desonide,
- (b) about 0.1% to 0.4% w/w of the polymer,
- 5 (d) about 3.0% to 10% w/w of a water miscible solvent,
- (e) less than about 0.25% w/w of a preservative, and
- (g) QSAD purified water to 100% w/w.

21. The composition of Claim 19 having a pH of about 5.0 to 6.0, which composition consists essentially of:

- 10 (a) about 0.025 to 0.05% w/w desonide,
- (b) about 0.3% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 5% w/w propylene glycol,
- (e) about 0.2 – 0.25% w/w methylparaben and propylparaben, and
- 15 (g) QSAD purified water to 100% w/w.

22. The composition of Claim 1 in combination with a container that accurately administers a portion of the composition for topical administration to a patient.

23. The composition of Claim 22 in combination with labeling instructions for use in treating the skin disorder.

- 20 24. A method for treating a skin disorder in a human subject, which method comprises topically administering a composition having a pH of about 3 to about 9 and a viscosity of less than about 15,000 cP to an affected area of the subject's skin having such disorder in an amount and for a period of time sufficient to improve the skin disorder, wherein the composition consists essentially of

- (a) a therapeutically-effective amount of at least one compound useful for treating such disorder,
 - (b) a pharmaceutically-acceptable lightly cross-linked polyacrylic acid polymer compatible with the pharmaceutical active material,
 - 5 (c) a pharmaceutically-acceptable base to adjust pH
 - (d) optionally a water miscible solvent,
 - (e) optionally a preservative,
 - (f) optionally an oil phase in combination with a surfactant, and
 - (g) water.
- 10 25. A method of preparing a composition having a viscosity of less than about 15,000 cP and a pH of about 3 to 9 useful for treating a skin disorder in a human subject, which method comprises
- (a) combining water with a therapeutically-effective amount of at least one compound useful for treating such disorder and a pharmaceutically-acceptable, lightly cross-linked polyacrylic acid polymer compatible with the compound,
 - 15 (b) adjusting the pH to about 3 to 9, and
 - (c) optionally combining a water-miscible solvent, a preservative, and at least one surfactant alone or in combination with an oil phase component to form the composition.
- 20 26. The method of Claim 25, wherein the compound is an antibiotic, imidazole, retinoid, corticosteroid, or a nonsteroidal anti-inflammatory drug (NSAID).
27. The method of Claim 26, wherein the compound is an antibiotic alone or in combination with a corticosteroid or a retinoid.
- 25 28. The method of Claim 27, wherein the compound is an antibiotic alone.
29. The method of Claim 28, wherein the antibiotic is clindamycin phosphate.
30. The method of Claim 29, wherein the composition has a pH of about 4 to 7, and consists essentially of

- (a) about 0.5% to 2.0% w/w clindamycin phosphate,
- (b) about 0.1% to 0.4% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 15.0% to 25.0% w/w of a water miscible solvent,
- 5 (e) less than about 0.2% w/w of a preservative, and
- (g) QSAD purified water to 100% w/w.

31. The method of Claim 30, wherein the water miscible solvent of part (d) is present in an amount of about 3.0% to 10.0% w/w.

32. The method of Claim 30, wherein the composition has a pH of about 5 to 6, and
10 consists essentially of:
- (a) 1.0 to 1.5% w/w clindamycin phosphate,
 - (b) 0.2% w/w of the polymer,
 - (c) the base to adjust pH,
 - (d) 15.0% w/w propylene glycol and 5.0% w/w polyethylene glycol 400
 - 15 (e) 0.1 – 0.15% w/w methylparaben, and
 - (g) QSAD purified water to 100% w/w.

33. The method of Claim 6 having a pH of about 5.0 to 6.0, wherein the composition
consists essentially of:

- (a) about 1.0 to 1.5% w/w clindamycin phosphate,
- 20 (b) about 0.2% w/w of the polymer,
- (c) the base to adjust pH,
- (d) about 5.0% w/w polyethylene glycol 400
- (e) about 0.1 – 0.15% w/w methylparaben, and
- (g) QSAD purified water to 100% w/w.

- 25 34. The method of Claim 27, wherein the antibiotic is combined with a corticosteroid, the antibiotic is clindamycin phosphate, and the corticosteroid is desonide, hydrocortisone valerate, fluocenolone acetinide, hydrocortisone butyrate, or triamcinolone acetonide.

35. The method of Claim 27, wherein the antibiotic is combined with a retinoid, the antibiotic is clindamycin phosphate, and the retinoid is tretinoin.

36. The method of Claim 35, wherein the composition is a gel having a pH of about 4 to 7 and consists essentially of

37. The method of Claim 35, wherein the composition is a lotion having a pH of about 5 to 6 and consists essentially of

- 15 (a) (i) about 0.5% to about 2.0% w/w clindamycin phosphate and
(ii) about 0.01% to about 0.05% w/w tretinoin;
(b) about 0.1% to about 0.5% w/w of the polymer;
(c) the base to adjust pH;
(d) about 5% to about 30% w/w of a water-miscible solvent;
(e) less than about 0.2% of a preservative; and
20 (f) an oil phase in combination with at least one surfactant to form an emulsion; and
(g) QSAD purified water 100% w/w.

38. The method of Claim 26, wherein the compound is a NSAID.

39. The method of Claim 38, wherein the compound is naproxen or diclofenac or a
25 pharmaceutically-acceptable salt thereof.

40. The method of Claim 26, wherein the composition has a corticosteroid as the sole active agent.

41. The method of Claim 40, wherein the corticosteroid is diflorasone diacetate, desonide, fluticasone propionate, halobetasol propionate, or budesonide.
 42. The method of Claim 41, wherein the corticosteroid is halobetasol propionate.
 43. The method of Claim 41, wherein the corticosteroid is desonide.
- 5 44. The method of Claim 43, wherein the composition exhibits having a pH of about 4.0 to 7.0 and consists essentially of
- (a) about 0.01% to 0.1% w/w desonide,
 - (b) about 0.1% to 0.4% w/w of the polymer,
 - (d) about 3.0% to 10% w/w of a water miscible solvent,
- 10 (e) less than about 0.25% w/w of a preservative, and
- (g) QSAD purified water to 100% w/w.
45. The method of Claim 43, wherein the composition exhibits having a pH of about 5.0 to 6.0 and consists essentially of
- 15 (a) about 0.025 to 0.05% w/w desonide,
- (b) about 0.3% w/w of the polymer,
 - (c) the base to adjust pH,
 - (d) about 5% w/w propylene glycol,
 - (e) about 0.2 – 0.25% w/w methylparaben and propylparaben, and
- (g) QSAD purified water to 100% w/w.
- 20 46. The method of Claim 25, which method further comprises placing the composition in a container from which drops are accurately administered for topical administration to a patient.
47. The method of Claim 46, which method further comprises combining the container with labeling instructions for use in treating the skin disorder.

INTERNATIONAL SEARCH REPORT

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| International application No. PCT/US01/25841 |
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| A. CLASSIFICATION OF SUBJECT MATTER | | |
| IPC(7) : A61K 7/00, 81/76; A61N 95/56 US CL : 424/401, 404, 78.02, 78.03, 78.05 According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED | | |
| Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/401, 404, 78.02, 78.03, 78.05 | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) NONE | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X,P | US 6,106,848 A (PREUILH et al) 22 August 2000 (22.08.2000), abstract, Columns 1-5, Examples 1-2. | 1-47 |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | |
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| (54) Title: NONSYMBIOTIC PLANT HEMOGLOBINS TO MAINTAIN CELL ENERGY STATUS | | | | | |
| <p>A. pAS1 (Sense)</p> | | | | | |
| <p>B. pAS2 (Anti-sense)</p> | | | | | |
| (57) Abstract | | | | | |
| <p>Nonsymbiotic hemoglobins are broadly present across evolution; however, the function of these proteins is unknown. Cultured maize cells have been transformed to constitutively express a barley hemoglobin gene in either the sense (HB^s) or antisense (HB^a) orientation. Hemoglobin protein in the transformed cell lines was correspondingly higher or lower than in wild type cells under normal atmospheric conditions. Limiting oxygen availability, by placing the cells in a nitrogen atmosphere for 12 hours, had little effect on the energy status of cells constitutively expressing hemoglobin, but had a pronounced effect on both wild type and HB^s cells, where ATP levels declined by 27 % and 61 % respectively. Energy charge was relatively unaffected by the treatment in HB^s and wild type cells, but was reduced from 0.91 to 0.73 in HB^a cells suggesting that the latter were incapable of maintaining their energy status under the low oxygen regime. Similar results were observed with <i>P. aeruginosa</i> cells transformed with an hb expression vector. It is suggested that nonsymbiotic hemoglobins act to maintain the energy status of cells in low oxygen environments and that they accomplish this effect by promoting glycolytic flux through NADH oxidation, resulting in increased substrate level phosphorylation. Nonsymbiotic hemoglobins are likely ancestors of an early form of hemoglobin that sequestered oxygen in low oxygen environments, providing a source of oxygen to oxidize NADH to provide ATP for cell growth and development. This in turn suggests that cells containing increased levels of hb protein will survive longer under low oxygen tension or high energy demand.</p> | | | | | |

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HEMOGLOBINS TO MAINTAIN CELL ENERGY STATUS

The present invention relates generally to the field of expression vectors and transgenic organisms.

BACKGROUND OF THE INVENTION

Hemoglobins are widespread throughout the biosphere (Wittenberg and Wittenberg, 1990, *Annu Rev Biophys Biophys Chem* 19:217-241). They are found in a broad range of organisms from bacteria, through unicellular eukaryotes, to plants and animals, suggesting that they predate divergence of life into plant and animal forms. Plant hemoglobins have been classified into symbiotic and nonsymbiotic types (Appleby, 1992, *Sci Progress* 76:365-398): symbiotic hemoglobins are found in plants that are capable of participating in microbial symbioses, where they function in regulating oxygen supply to nitrogen fixing bacteria; nonsymbiotic hemoglobins have only recently been discovered and are thought to be the evolutionary predecessors of the more specialized symbiotic leghemoglobins. The ubiquitous nature of nonsymbiotic hemoglobins is evidenced by their broad presence across the plant kingdom (Appleby, 1985, Nitrogen Fixation and CO₂ Metabolism, eds. Ludden and Burris, pp. 41-51) and the widespread presence and long evolutionary history of plant hemoglobins suggest a major role for them in the life of plants.

Specifically, plant hemoglobins have been known to exist in the root nodules of legumes for almost 60 years (Kubo, 1939, *Acta Phytocem* 11:195-200; Keilen and Wang, 1945, *Nature* 155:227-229). Over the years, hemoglobins have been positively identified in three non-leguminous dicotyledonous plants: *Parasponia andersonii*, *Tream tomentosa*, and *Casuarina glauca* (Appleby et al., 1983, *Science* 220:951-954; Bogusz et al., 1988, *Nature* 331:178-180; Kortt et al., 1988, *FEBS Lett* 180:55-60). Recently, an Hb cDNA from barley was isolated and the gene was demonstrated to be expressed in seed and root tissues under anaerobic conditions (Taylor et al., 1994, *Plant Mol Biol* 24:853-862), providing further evidence to support the contention that plant hemoglobins have a common origin (Landsmann et al., 1986, *Nature* 324:166-168). Since Hb has now been demonstrated to occur in two of the major divisions of the plant kingdom, it is likely

that an Hb gene is present in the genome of all higher plants (Brown et al., 1984, *J Mol Evol* 21:19-32; Bogusz et al., 1988; Appleby, 1992, *Sci Progress* 76:365-398; Taylor et al., 1994; Andersson et al., 1996, *Proc Natl Acad Sci USA* 93:427-431; Hardison, 1996, *Proc Natl Acad Sci USA* 93:5675-5682).

Very little, however, is known about the function of Hb, although it has been proposed that nonsymbiotic hemoglobins may act either as oxygen carriers to facilitate oxygen diffusion, or oxygen sensors to regulate expression of anaerobic proteins during periods of low oxygen supply. The proteins from barley (Duff et al, 1997, *J Biol Chem* 272:16746-16752, incorporated herein by reference) and rice (Arredondo-Peter et al, 1997, *Plant Physiol* 115:1259-1266) and AHB1 from *Arabidopsis* (Trevaskis et al, 1997, *Proc Natl Acad Sci* 94:12230-12234) have been shown to have high oxygen avidity, with dissociation constants for oxyhemoglobin of 2.86 nM, 0.55 nM and 1.6 nM respectively, resulting in conditions whereby the free protein will remain oxygenated at oxygen concentrations far below those at which anaerobic processes are activated. Thus, while roles for Hb in the facilitated diffusion and sensing of oxygen have been proposed (Appleby, 1992), it is unlikely that these hemoglobins would function as either facilitators of oxygen diffusion or sensors of oxygen, unless the oxygen avidity was modified by interaction with another component within the cell. Thus, while Hb or Hb related proteins are found in all divisions of living organisms, their function has not been well defined.

Herein, it is shown that nonsymbiotic hemoglobins function to maintain the energy status of cells exposed to low oxygen tensions and that this property may be a common feature throughout evolution, either during exposure to hypoxia or under high energy demand.

SUMMARY OF THE INVENTION

According to one aspect of the invention there is provided a recombinant expression system capable, when transformed into an organism, of expressing a gene encoding a nonsymbiotic hemoglobin, which system comprises a nucleotide sequence encoding said nonsymbiotic hemoglobin operably linked to control sequences effective in said organism.

The control sequences may include a strong constitutive promoter.

The nonsymbiotic hemoglobin may be barley hemoglobin.

The organism may be a plant. The plant may be maize.

Preferably, the promoter is maize ubiquitin promoter.

The organism may be a bacteria. The bacteria may be an obligate aerobe. The obligate aerobe may be *P. aeruginosa*.

According to a second aspect of the invention, there are provided cells transformed with any one of the expression systems described above.

According to a third aspect of the invention, there is provided a transgenic organism whose genome has been modified to contain the expression system described above.

According to a fourth aspect of the invention, there is provided a method of increasing tolerance to hypoxic conditions comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

placing the organism under hypoxic conditions,

wherein the oxygen-binding protein acts to maintain cellular energy status during the hypoxic conditions by making oxygen available for cellular metabolism at low oxygen tension.

According to a fifth aspect of the invention, there is provided a method of lowering the level of fermentation products in an organism comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

reducing the level of fermentation products in the cells of the organism by maintaining cell energy status such that fermentation is bypassed.

According to a sixth aspect of the invention, there is provided a method of maintaining cellular metabolism under hypoxic conditions comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

placing the organism under hypoxic conditions,

wherein the oxygen-binding protein acts to maintain cellular

metabolism status by providing oxygen for cellular metabolism.

According to a seventh aspect of the invention, there is provided a method of increasing oxygen uptake of an organism comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

exposing the organism to an oxygen-containing environment,

wherein the increased cellular levels of the oxygen-binding protein results in increased oxygen uptake.

According to an eighth aspect of the invention, there is provided a method of improving the agronomic properties of a plant comprising:

providing a plant having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

growing the plant.

The improved agronomic properties may include germination, seedling vigour, reduced cellular levels of fermentation products, increased oxygen uptake, and increased tolerance to hypoxic conditions.

According to a ninth aspect of the invention, there is provided a method of performing skin grafts comprising:

isolating skin cells from a patient;

transfected the skin cells with an expression system comprising a nucleotide sequence encoding an oxygen binding protein having a low dissociation constant for oxygen operably linked to control sequences effective in skin cells;

culturing the skin cells such that the oxygen binding protein is expressed; and

grafting the skin cells onto a region of skin tissue attached to the patient.

According to a tenth aspect of the invention, there is provided a method of transplanting an organ from a donor to a recipient comprising:

providing an organ for transplant;

infusing the organ with an oxygen binding protein having a low dissociation constant for oxygen, thereby improving oxygen supply to the organ; and

transplanting the organ into the recipient.

The oxygen binding protein having a low dissociation constant for oxygen described in the above methods may be a nonsymbiotic hemoglobin. The nonsymbiotic hemoglobin may be barley hemoglobin.

According to an eleventh aspect of the invention, there is provided a method of selecting seeds for breeding to produce seed lines having desirable characteristics comprising:

- providing a representative seed of a given seed line;
- growing the seed such that the seed germinates;
- isolating an extract from the seed;
- measuring levels of hemoglobin expression within the extract; and
- selecting or rejecting the seed for further breeding based on the hemoglobin levels.

According to a twelfth aspect of the invention there is provided a method of determining if a seed is germinating comprising:

- providing a seed suspected of germinating;
- isolating an extract from the seed; and
- measuring levels of hemoglobin expression within the extract,

wherein high levels of hemoglobin expression indicate that the seed is germinating.

One embodiment of the invention will now be described in conjunction with the accompanying figures in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram summarizing the structures of pAS1 and pAS2 respectively.

Figure 2 is the protein immunoblot analysis of hemoglobin expression in wild-type (BMS), HB⁺ and HB⁻ maize cell lines with recombinant barley hemoglobin-specific antibody.

Figure 3 is a graph of the growth rate of wild-type (BMS), HB⁺ and HB⁻ maize cell lines under normal atmospheric conditions.

Figure 4 is a bar graph comparison of oxygen uptake by maize wild-

type (BMS), HB⁺ and HB⁻ cells.

Figure 5 is a bar graph comparison of ATP levels in wild-type (BMS), HB⁺ and HB⁻ maize cells grown under normal atmospheric conditions, after 12 hours of treatment with nitrogen, under normal atmospheric conditions following treatment with Antimycin A and after 12 hours of treatment with nitrogen following treatment with Antimycin A.

Figure 6 is a bar graph comparison of CO₂ evolution by maize cells cultured under a nitrogen atmosphere.

Figure 7 is a graph of alcohol dehydrogenase activity in maize cells cultured under a nitrogen atmosphere.

Figure 8 is a bar graph of oxygen uptake by maize cells under low oxygen atmosphere.

Figure 9 is a bar graph of oxygen uptake by maize cells under normal air conditions.

Figure 10 is a graph of cell culture growth following hypoxic treatment.

Figure 11 is a bar graph of the amount of hemoglobin in crude extracts made from germinating barley seeds.

Figure 12 is a Western blot of proteins from transformed and wild type *P. aeruginosa*. Each lane consisted of 80 μg of crude protein extract from *P. aeruginosa* cells and the blot was probed with affinity purified barley Hb antibodies. Lane 1 contains protein extracted from bacteria transformed with the Hb expression vector, whereas Lane 2 contains protein extracted from wild-type bacteria.

Figure 13 is a Northern blot of RNA extracted over time from a germinating seedling.

Table 1 is a summary of measurements of energy charge and total adenylates in maize cells before and after exposure to a nitrogen atmosphere for 12 hours.

Table 2 is a summary of A₆₀₀ measurements of transformed and untransformed *E. coli* and *P. aeruginosa* cells grown aerobically or anaerobically. Measurements are the averages of two separate determinations which did not vary

by more than 15%.

Table 3 is a summary of ATP measurements of transformed and untransformed *E. coli* and *P. aeruginosa* cells grown aerobically and anaerobically. Measurements are the results of duplicate assays from three separate experiments. Standard error in all cases was no greater than 10%.

DETAILED DESCRIPTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

Expression plasmids containing DNA encoding a nonsymbiotic hemoglobin were constructed. These plasmids also included a strong constitutive promoter and a selectable marker compatible with the specific host organisms such that when these plasmid constructs were transformed into the host organisms, the constructs expressed elevated levels of Hb protein compared to wild type cells. In all cases, the transformed cells had an elevated level of ATP. This strongly suggests that nonsymbiotic hemoglobin functions in maintaining ATP levels and is involved in primary energy metabolism. Thus, cells engineered to express a higher level of Hb will survive longer under low oxygen tension or high energy demand. In other words, the cells maintain vigour and hardiness under stressful conditions and can better adapt to varying growth conditions. That is, transformed crop plants containing elevated levels of the nonsymbiotic hemoglobin gene may exhibit increased crop yields due to the ability of the plant to more effectively survive periods of flooding, the ability of the seed and seedling to develop more vigorously under adverse germination and/or growth conditions, and the ability of winter crops to survive ice cover more effectively. Furthermore, given that the effect of nonsymbiotic hemoglobin on cell energy status is seen in both bacteria and plants, it seems likely that this phenomenon is universal. This would in turn mean that nonsymbiotic hemoglobins have potential applications in a

number of medical procedures. For example, skin cells from burn victims are frequently cultured for transplantation back to the burn victim. Given that oxygen supply is a limiting factor for growth and survival of the transplanted skin grafts, skin cells transfected with nonsymbiotic hemoglobin may possess improved growth and survival. Similarly, oxygen supply is also a limiting factor in other medical procedures, for example, organ transplants. That is, it is likely that organs possessing nonsymbiotic hemoglobins may have enhanced survival following transplant. Furthermore, the hemoglobin gene itself is shown to be expressed at time of germination, meaning that the hemoglobin gene may be used as a marker for germination and also as a marker for breeding. That is, levels of hemoglobin in specific seed lines may be used to select seeds for developing progeny seeds capable of expressing either higher or lower levels of hemoglobin.

In one embodiment, expression plasmids containing DNA encoding barley hemoglobin in both the sense and anti-sense orientation were constructed. The plasmids also included the maize ubiquitin promoter, and a selectable marker for selection of transformants, in this embodiment, a herbicide resistance gene (Bar), conferring resistance to glufosinate ammonium. The plasmids were transformed into cultured maize cells of the Black Mexican Sweet (BMS) variety, producing a cell line containing the sense plasmid (HB⁺) and a cell line containing the antisense plasmid (HB⁻).

When grown in an air environment, the HB⁺ and HB⁻ cells did not differ significantly from wild-type BMS cells in terms of growth rate, oxygen consumption or cellular ATP levels. However, when grown under a nitrogen atmosphere, ATP levels in the HB⁺ cells remained essentially the same as those observed under normal atmosphere conditions while ATP levels dropped significantly in wild-type and HB⁻ cells. Analysis of ATP levels in all three cell lines under a nitrogen atmosphere following treatment with Antimycin A (which blocks mitochondrial electron transport) indicated that the increase in ATP in HB⁺ cells was not cytochrome-mediated. Furthermore, measurements of CO₂ evolution and alcohol dehydrogenase activity in HB⁺ cells suggested lower ethanolic fermentation rates in this cell line.

These data indicate that over-expression of nonsymbiotic

hemoglobins helps maintain the energy status of cells grown at low oxygen tensions. This in turn has several possible applications, as cells capable of maintaining energy status at low oxygen tensions would have, for example, increased tolerance to a low oxygen atmosphere, improved germination rates and seedling vigour, increased ability to maintain cellular metabolism at low oxygen tension, reduced levels of fermentation products within the cells due to lowered alcohol dehydrogenase activity, increased oxygen uptake under low oxygen tension and increased tolerance to hypoxic conditions such as, for example, ice encasement, flood and growth in compacted soil.

EXAMPLE I – PLANT CELL CULTURES

Black Mexican Sweet (BMS) (wild-type), HB⁺ and HB⁻ maize cells were cultured in 250 ml flasks as cell suspensions in 50 ml of MS medium (Murashige and Skooge, 1962, *Physiol Plant* 15:473-497) macro and micro elements supplemented with thiamine 0.5 mg/litre, L-asparagine 150 mg/litre, 2,4-dichlorophenoxyacetic acid 2 mg/litre and sucrose 20 g/litre. Cultures were shaken at 150 rpm at 25°C. Cells were subcultured every 7 days. Nitrogen treatment was applied by replacing air in culture flasks with nitrogen and closing the flasks with rubber stoppers, otherwise culture flasks were closed with caps allowing for free exchange of air. Antimycin A was added as a 27 mM stock solution in 2-propanol to give a final concentration of 0.2 mM. Cell samples were collected by filtration. Cell samples used for adenylate measurements were immediately frozen in liquid nitrogen and stored at -80°C until used.

EXAMPLE II – CONSTRUCTION OF PLANT EXPRESSION VECTORS

Sall/NotI digested and end-filled barley hemoglobin cDNA was cloned into BamHI digested and end-filled pAHC17 plasmid (Christensen and Quail, 1996, *Transgenic Research* 5:213-218) in sense and antisense orientation to generate pAS1 (sense) and pAS2 (antisense) plasmids. An EcoRI digested, end-filled with synthetic HindIII linker, 1.35 kb 35S promoter -bar gene- 35S terminator fragment from pDB1 (Becker et al, 1994, *Plant J* 5:299-307) was inserted into HindIII digested pAS1 and pAS2, as described below.

EXAMPLE III – PLANT CELL TRANSFORMATION AND SELECTION

A silicon carbide fibres-mediated transformation system was used as described in Kaepller et al, 1992, *Theor Appl Genet* 84:560-566 to transform BMS maize cells with pAS1 and pAS2 vectors. Resistant colonies were selected on culture medium solidified with 0.2% Phytigel™ (Sigma) and supplemented with glufosinate ammonium at a concentration of 5 mg/litre.

EXAMPLE IV – PLANT PROTEIN IMMUNOBLOTS

SDS gel electrophoresis, protein transfer to nitrocellulose membrane and antibody detection were performed according to standard Bio-Rad protocol (Bio-Rad bulletin 1721). Hemoglobin protein in transformed lines was detected by immunoblots, using a polyclonal antibody raised against barley recombinant hemoglobin. Protein concentration was calculated by densitometric comparison of immunoblots (in four repetitions) with a standard curve of known concentrations of recombinant hemoglobin using a Sharp Diversity 1 PDI-325OE Scanner™.

EXAMPLE V – MEASUREMENT OF PLANT GROWTH PARAMETERS

Culture growth was measured by sedimentation in 25 ml graduated pipettes. Adenylates were extracted in 1N perchloric acid from frozen cell samples at -10°C and ATP, ADP and AMP assayed spectrophotometrically by established protocols as described in Lowry and Passonneau, 1972, A Flexible System of Enzymatic Analysis, Academic Press: New York.

Alcohol dehydrogenase activity was measured in the ethanol – acetaldehyde direction in fresh cell extracts. Enzyme extraction and spectrophotometric measurements were performed as described in Hanson and Jacobsen, 1984, *Plant Physiol* 75:566-572.

For measurements of CO₂ evolution from cell cultures, 1 ml gas samples were collected with an air tight syringe, from stoppered culture flasks, and analyzed by gas chromatography (Shimadzu GC-8AIT™).

Oxygen uptake was measured polarographically with an O₂ electrode (Rank Brothers, Cambridge, UK) for 5 to 30 minutes. The incubation cell contained

2 ml of culture medium, 0.2 ml (sedimented cell volume) of cells. In some measurements, 0.2 mM Antimycin A was added, as described below.

EXAMPLE VI – EFFECT OF NONSYMBIOTIC HEMOGLOBIN ON PLANT CELL ENERGY STATUS

As noted above, cultured maize cells of the Black Mexican Sweet (BMS) variety were transformed with a barley hemoglobin gene to observe the effect of increasing or decreasing hemoglobin expression on cell metabolism. Specifically, transformation vectors, shown in Figure 1, were prepared containing the open reading frame of a barley hemoglobin cDNA in sense and antisense orientations, which were placed under the control of a strong constitutive promoter, in this embodiment, the maize ubiquitin (*Ubi1*) promoter. A herbicide resistance gene (Bar), conferring resistance to glufosinate ammonium, was cloned head to tail with the hemoglobin gene constructs to enable selection of transformed cell lines. Twenty-four independently transformed sense (pAS1) and thirty-eight anti-sense (pAS2) lines were obtained. Transformation was confirmed by Southern blot analysis and PCR. A sense line (HB⁺) expressing hemoglobin at levels 10 fold higher than wild type (BMS) and an antisense line (HB⁻) with 10 times lower expression of hemoglobin than BMS, as shown in Figure 2, were selected for further studies, as described below.

The three cell lines, grown in an air environment, did not differ significantly from one another with respect to culture growth rates, as shown in Figure 3, and consumption of oxygen, as shown in Figure 4. Furthermore, steady state ATP levels were essentially the same in the three types of cells, as shown in Figure 5. However, after incubation of the cells for a further 12 hours under an atmosphere of nitrogen gas, significant differences were observed in the ATP levels of the cell types. Specifically, the level of ATP was highest in HB⁺ cells, being only marginally lower than under normal atmospheric conditions while ATP levels in wild type (BMS) cells were 27% lower than HB⁺ cells and ATP levels in HB⁻ cells were 61% lower than HB⁺ cells. Differences in energy charge and total adenylates were also observed in cells exposed to nitrogen atmospheres, as summarized in Table 1. As can be seen, energy charge was relatively the same in

all three cell types under normal atmospheric conditions and in BMS and HB⁺ cell lines after 12 hours of a nitrogen atmosphere. HB⁻ cells, on the other hand, were unable to maintain energy charge during the 12 hour exposure to a nitrogen atmosphere. Total adenylates remained the same in all three cell lines under atmospheric conditions and in HB⁺ cells in a nitrogen atmosphere; however, in BMS and HB⁻ cells, the total adenylates declined by about 35 percent.

From this, it is evident that determining what part of the cell's metabolism contributes to this increased ability to maintain energy status in the presence of hemoglobin is critical to understanding the role of nonsymbiotic hemoglobin. To examine the possibility that hemoglobin might provide oxygen to generate ATP via cytochrome-mediated respiratory processes, Antimycin A (0.2 mM), which blocks mitochondrial electron transport in the span from cytochrome b to c and has been shown to induce hemoglobin expression in aleurone layers (Nie and Hill, 1997, *Plant Physiol* 114:835-840) was used. Antimycin A inhibited 80% of the oxygen uptake by maize cells within 30 minutes of treatment. After 12 hours exposure to Antimycin A in an air environment, ATP levels in the three cell types were similar to those of untreated cells after 12 hours under a nitrogen atmosphere, as shown in Figure 5. However, upon placing Antimycin A-treated cells in a nitrogen atmosphere for 12 hours, the cell lines all showed decreases in ATP but, consistent with the previous experiments, the levels of ATP decreased in the order HB⁺, BMS, and HB⁻. This provides evidence that the increase in ATP brought about by the presence of hemoglobin was not the result of cytochrome-mediated mitochondrial respiration. It is also unlikely that the increased ATP is the result of oxyhemoglobin supporting mitochondrial alternative oxidase activity, which would increase substrate phosphorylation through glycolysis.

Furthermore, as shown in Figure 6, CO₂ evolution from hypoxic HB⁺ cells was 20 to 30% lower than CO₂ levels evolved from BMS or HB⁻ cells, which would not be anticipated if the Krebs cycle was being maintained through alternative oxidase activity.

EXAMPLE VII – PLANT CELL ALCOHOL DEHYDROGENASE LEVELS

An examination of alcohol dehydrogenase activity (ADH) in the cell

lines showed that ADH increased in all three lines over the course of the experiments, but the ADH activity was significantly lower in the sense transformants (HB⁺) than in antisense transformants (HB⁻) or wild-type cells, as shown in Figure 7. Fluorescein diacetate staining (Heslop-Harrison et al, 1984, *Theor Appl Genet* 67:367-375) showed no difference in the viability of the cell lines at the end of the incubation period. The reduced ADH activity, along with lower CO₂ evolution in HB⁺ cells, likely reflects lower ethanolic fermentation rates, suggesting that a fermentative pathway may be the main source of carbon dioxide production in this system.

EXAMPLE VIII – OXYGEN UPTAKE BY PLANT CELLS

As discussed above, the presence of nonsymbiotic hemoglobin clearly affects the energy status of maize cells under hypoxia. Furthermore, differences between the HB⁺, wild type and HB⁻ cells were observed only under the conditions of limited oxygen. To investigate the possibility that the observed differences may be due to the different abilities of the cell lines to utilize oxygen that is available in low concentrations, the oxygen uptake by the maize cells was measured under normal air conditions, shown in Figure 9, and in medium equilibrated with a mixture of 2% O₂ and 98% N₂, shown in Figure 8. Specifically, oxygen uptake was measured polarographically with an O₂ electrode. As can be seen, HB⁺ cells were more efficient at oxygen uptake than the wild-type cells and much more efficient than the HB⁻ cells. Specifically, the oxygen uptake by the HB⁺ cells from the medium equilibrated with 2% oxygen was 55% of that of all three cell lines under normal air conditions, as shown in Figures 8 and 9. Furthermore, wild-type BMS and HB⁻ cells grown at 2% O₂ exhibited O₂ uptake at 44% and 18% respectively of the oxygen uptake of the cell lines grown under normal conditions, as shown in Figures 8 and 9. These results clearly indicate that the rate of oxygen utilization by maize cells under low oxygen atmosphere depends on the presence of the non-symbiotic hemoglobin.

EXAMPLE IX – PLANT CELL GROWTH AFTER EXPOSURE TO HYPOXIC STRESS

The ability of the cell cultures to continue growth after exposure to hypoxic stress was also tested. Maize cell cultures were placed under the atmosphere of nitrogen for 12 and 24 hours, then cells were harvested, transferred to a fresh medium and their growth was monitored by sedimented cell volume measurements, as shown in Figure 10. Upon placement under the N₂ atmosphere, the cell growth of all three cell lines ceased, but resumed after transfer to the fresh medium and normal atmospheric conditions. However, while the HB⁺ cell cultures resumed growth almost immediately after the transfer to normal air conditions, the HB⁻ cells showed a 36 hour lag period before commencement of intensive growth. Furthermore, the growth of the wild-type cultures, during the first 36 hours after the transfer to normal conditions, was slower than that of HB⁺ cells, as shown in Figure 10. It is of note that after the initial 36 hour period, the growth rates of the three cell lines were almost identical. The differences in cell volume at each time point were most likely a result of the growth activity during this initial period. The culture re-growth after the 24 hour hypoxic exposure was the same for all three cell lines, as after the 12 hour treatment. The observed differences may be explained by different levels of cell survival under stress, and, depending on the cell line, the same cell volume could contain different numbers of growing cells. On the other hand, the increased growth rates of the HB⁻ and the wild-type BMS cultures after a lag period, shown in Figure 10, suggests a longer stress recovery period rather than cell death.

EXAMPLE X – HEMOGLOBIN EXPRESSION IN GERMINATING BARLEY

Polyclonal antibodies to purified recombinant barley hemoglobin were raised in rabbits and used to investigate the expression of hemoglobin in monocotyledonous plants. Specifically, hemoglobin was shown to be expressed in whole seeds, as shown in Figure 11, embryo-less half seeds and excised embryos during germination. The fact that hemoglobin was expressed in both embryo-less half seeds and excised embryos indicates that the gene is independently responsive to signals in both tissues and suggests that both the aleurone layer and the embryo may experience oxygen deficiencies during the imbibition process. In the excised embryo, hemoglobin was induced between 4 and 6 hours after

imbibition. Since germination and the early stages of seedling growth are known to be periods of high metabolic demand (Bewley and Black, 1990, *Prog Nucleic Acid Res Mol Biol*, 38:165-193, incorporated herein by reference), this data is consistent with the proposed concept that a demand on energy charge or ATP requirement is primarily responsible for hemoglobin induction (Nie and Hill, 1997, *Plant Physiol* 114:835-840). Major changes in ATP content of the embryos did occur within one hour after imbibition, which is consistent with previous reports. Protein hydration, protein synthesis and nucleotide synthesis are among the first events of germination. These early events, which consume large amounts of ATP, may well be a factor in the observed induction of hemoglobin synthesis at 4 to 6 hours after imbibition. However, induction occurs well before the major increase in α -amylase secretion, a period of high metabolic demand, and so the relationship between hemoglobin synthesis and energy availability needs further clarification.

In half seeds, there is an apparent induction of hemoglobin during imbibition, without the use of gibberellic acid to stimulate the synthesis of hydrolytic enzymes. Furthermore, isolated aleurone layers do not show appreciable amounts of hemoglobin unless induced by anoxia using a nitrogen environment (Nie and Hill, 1997). The aleuronales in these half-seeds may well be experiencing anoxia due to entrapment in the endosperm and seed coat.

Thus, to summarize, very little or no hemoglobin expression was observed in dry barley seeds but germination resulted in the expression of hemoglobin which peaked at 2-3 days after imbibition, as shown in Figure 11. Furthermore, hemoglobin expression was also observed in maize, wheat, wild oat and *Echinochloa crus gali* seeds during germination. Dissection of tissues from the barley seedlings showed that most of the hemoglobin was expressed in the root and seed coat (aleurone layer), with very little in the coleoptile. Imbibition of half seeds or excised embryos resulted in the expression of hemoglobin. ATP measurements of barley embryos showed that ATP levels quickly increased after imbibition. α -Amylase activity was also determined in the embryos to correlate hemoglobin expression with a well-characterized germination response. The results demonstrate that hemoglobin expression is a normal consequence of germination.

In addition, whole barley seeds were imbibed for 16 hours at 22°C. Embryos were excised from the caryopsis after 2, 4, 8, 10, 12, 14 and 16 hours imbibition. It was noted that radicle protrusion occurs after 8 hours. The embryos were ground in liquid nitrogen and RNA extracted for Northern analysis using an RNA probe transcribed from barley Hb cDNA. As can be seen in Figure 13, it was found that no message was present in unimbibed seeds but was detectable after just two hours imbibition. Expression increased up until 8 hours when radicle emergence occurred. The amounts of message then decreased for the next 8 hours. These experiments show that hemoglobin expression occurs during germination. As such, it is clear that hemoglobin expression can be used as a marker for germination.

EXAMPLE XI – CONSTRUCTION OF BACTERIAL EXPRESSION CONSTRUCTS

A recombinant Hb cDNA-containing pUC19 construct (Duff et al, 1997) was used as the starting material. The Hb cDNA was excised from the pUC19 construct by digestion with the restriction enzymes EcoRI and HindIII. The insert was then ligated into the pPZ375 multiple cloning site between HindIII and EcoRI such that the coding sequence was in the correct reading frame.

EXAMPLE XII – TRANSFORMATION AND SCREENING OF RECOMBINANT *E. COLI*

Escherichia coli DH5 α cells were then transformed with the pPZ375-Hb construct according to the instructions for the Canadian Life Technologies subcloning efficiency competent cells, incorporated herein by reference. It is of note that in this instance Blue-White screening was unnecessary. *E. coli* cells were plated, screened and grown as previously described (Duff et al, 1997). Plasmid DNA was prepared from the cells using the small scale preparation protocol (Sambrook et al, 1989). The recombinant plasmid was then used to transform competent *Pseudomonas aeruginosa*, as described below.

EXAMPLE XIII – PREPARATION AND TRANSFORMATION OF COMPETENT *PSEUDOMONAS AERUGINOSA*

100 ml of LB media in a 500 ml flask was inoculated with 1 ml of an overnight culture of *Pseudomonas aeruginosa* and grown for 2.5 hours to a cell density of approximately 10^8 cells/ml. Cells were harvested by centrifugation at 1000 g and then resuspended in 10 ml of Competency Buffer (0.15 M MgCl₂, 15% (v/v) glycerol, 10 mM Pipes (Sigma), pH 7.0). Cells were incubated in an ice water bath for 5 minutes, pelleted at 1000 g, and resuspended in 10 ml of Competency Buffer. Cells were then incubated in an ice water bath for 20 minutes, pelleted at 1000 g, and resuspended in 10 ml of Competency Buffer. Cells were then frozen at -70°C until used for transformation. DNA (approximately 0.2 µg of the recombinant plasmid) was used to transform 200 µl of competent *Pseudomonas aeruginosa* cells. Cells were incubated in an ice water bath for 60 minutes and heat shocked for 3 minutes at 37°C while gently rocking the tube. Cells were placed in an ice water bath for 5 minutes. 0.5 ml of room temperature LB broth was added and the cells were incubated at 37°C for 2.5 hours with no rotation. Cells were concentrated by centrifugation and plated on appropriate media.

EXAMPLE XIV – ELECTROPHORESIS AND BACTERIAL PROTEIN IMMUNOBLOTTING

DNA agarose electrophoresis, protein acrylamide electrophoresis and protein immunoblotting was performed as previously described above.

EXAMPLE XV – BACTERIAL GROWTH AND TREATMENT

E. coli was inoculated into four 400 ml cultures and grown for 3 hours. After 3 hours, A₆₀₀ was measured as an estimate of bacterial growth and then either air or nitrogen was bubbled through the media for 5 minutes and the flasks were sealed. The bacteria were grown for a further 6 hours after which the A₆₀₀ was determined for each flask as an estimate of bacterial growth. Similarly, *P. aeruginosa* was inoculated into four 400 ml cultures and grown for 3 hours using the same protocol as described above for *E. coli*,

EXAMPLE XVI – ATP EXTRACTION AND ASSAY

ATP was extracted and assayed according to standard procedures

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known in the art (Lowry and Passonneau, in A Flexible System of Enzymatic Analysis (1972, Academic Press: New York) pp 146-222, incorporated herein by reference).

EXAMPLE XVII – EXPRESSION OF BARLEY Hb IN *E. COLI* AND *P. AERUGINOSA*

Untransformed *E. coli* cells and *E. coli* cells previously transformed with Hb cDNA were used (Duff et al., 1997). Western blot analysis confirmed that both *E. coli* (data not shown) and *P. aeruginosa* (Figure 12) had been successfully transformed and were expressing significant amounts of Hb. Recombinant *E. coli* and *P. aeruginosa* were also visually more red than their wild type counterparts (data not shown). Levels of recombinant barley hemoglobin expressed in the two species of bacteria were roughly equal based on SDS-PAGE and protein immunoblot analysis.

EXAMPLE XVIII – GROWTH RATES OF *E. COLI* AND *P. AERUGINOSA*

The A_{600} measurements of 400 ml cultures of transformed and untransformed *E. coli* and *P. aeruginosa* grown under both aerobic and anaerobic conditions are shown in Table 2. *E. coli* containing the recombinant plasmid grew considerably slower than bacteria containing pUC19. There were no differences in growth between bacteria grown under air or anoxic conditions for *E. coli* containing either plasmid. *P. aeruginosa* containing the recombinant plasmid also grew somewhat slower than the bacteria containing pUC19. However, anoxic treatment virtually stopped the growth of both the wild type and recombinant obligate aerobic bacteria *P. aeruginosa*.

EXAMPLE XIX – ATP LEVELS IN *E. COLI* AND *P. AERUGINOSA*

ATP levels from aerobically and anaerobically grown *E. coli* and *P. aeruginosa* are shown in Table 3. As can be seen, *E. coli* cells had the same total ATP regardless of whether or not they were expressing barley Hb or whether they were grown under aerobic or non-aerobic conditions. However, *P. aeruginosa* containing the recombinant barley Hb had significantly higher levels of ATP under

both aerobic and non-aerobic conditions. These results are not surprising, given that *E. coli* readily adapts to grow in environments with limited oxygen. *P. aeruginosa*, on the other hand, is an obligate aerobe and is unable to grow in environments with limited oxygen. Furthermore, it is known that ATP levels and energy charge are directly related to the metabolic state of an organism and that organisms with low ATP levels and energy charge are generally considered to be under stress or in a state of dormancy. Thus, the fact that *P. aeruginosa* containing nonsymbiotic hemoglobin has an improved energy status is evidence that the presence of this protein facilitates adaptation to low oxygen tension.

DISCUSSION

Higher plant hemoglobins are cytoplasmic proteins (Wittenberg and Wittenberg, 1990). With this in mind, transformation constructs were designed for cytoplasmic expression of hemoglobin. Barley hemoglobin cDNA hybridizes to only one locus in barley and maize genomes (Taylor et al, *Plant Mol Biol* 24:853-862) and, therefore, sense and antisense expression of this cDNA would not be expected to affect the expression of any other genes. It is of note that the polyclonal anti-hemoglobin antibody used was raised and titrated against recombinant barley hemoglobin. Furthermore, it is clear that there is over and under expression of hemoglobin in the transgenic cells.

The lack of effect of hemoglobin on cell growth and oxygen uptake under normal air conditions likely reflects the fact that barley (Taylor et al, 1994) and maize hemoglobin genes are induced under conditions of limited oxygen availability, resulting in the protein having little effect when oxygen supplies are not impaired. The results, however, show clearly that the energy status of maize cells when oxygen is limiting is affected by the ability of the cells to produce hemoglobin. Total adenylates and ATP levels are maintained during the period of exposure to limiting oxygen when hemoglobin is constitutively expressed in the cells. Alternatively, when hemoglobin expression is suppressed by constitutive expression of antisense barley hemoglobin message, the cells are unable to maintain their energy status during oxygen limitation. In wild-type (BMS) cells, it would appear that the induction of native maize hemoglobin was sufficient to

maintain the energy charge, but not the total adenylate pool. This is consistent with the observation that a decline in the adenylate pool has been noted during hypoxia in maize root tips (Saint-Ges et al, 1991, *Eur J Biochem* 200:477-482). Under limiting oxygen, plant cells turn their metabolism towards fermentation in order to oxidize NADH necessary to maintain glycolytic substrate phosphorylation. Lower alcohol dehydrogenase activity in HB⁺ cells suggests that hemoglobin provides an alternative to potentially harmful fermentation. Specifically, carbon dioxide is produced by the HB⁺ cells in lower amounts than by HB⁻ and wild-type maize cells, reflecting lower ADH activity and suggesting that the ethanolic fermentation is the only source of CO₂. The dissociation constant of barley oxyhemoglobin is about 3 nM (Duff et al, 1997), indicating that oxyhemoglobin, acting alone, would be ineffective in providing oxygen to maintain mitochondrial respiratory processes. This is confirmed by the observation that Antimycin A has no effect on the ability of hemoglobin-containing cells in maintaining their energy status under low oxygen tensions. The results discussed above suggest that hemoglobin maintains energy status of the cell by means different from mitochondrial oxidative phosphorylation , probably by facilitating glycolysis to generate ATP through substrate level phosphorylation.

It is of note that hemoglobins of barley (Taylor et al, 1994) and maize as well as *Arabidopsis* AHB1 (Trevaskis et al, 1997) are hypoxia inducible. Furthermore, it has been demonstrated that, in barley hemoglobin, this is not due to a lack of oxygen per se, but in response to insufficient mitochondrial ATP synthesis. In addition, nonsymbiotic hemoglobins are expressed in metabolically active tissues such as roots (Taylor et al, 1994; Arredondo-Peter et al, 1997; Trevaskis, 1997), aleurone (Taylor et al, 1994), vascular tissues of leaves, stems and seedling cotyledons (Andersson et al, 1996, *Proc Natl Acad Sci* 93:5682-5687). Taken together, these data support a hypothesis that nonsymbiotic hemoglobins utilize available oxygen to maintain the cell's energy status in cells exposed to low oxygen tensions or other conditions that reduce cellular ATP levels. The very low dissociation constant of barley oxyhemoglobin makes it an ideal candidate for sequestering oxygen in low oxygen environments. Interaction with another compound, perhaps a flavoprotein, could create a complex capable of

oxidizing NADH, in a manner analogous to Hmp protein of *E. coli* (Poole et al, 1996, *Microbiology (Reading)* 142:1141-1148). This would provide an efficient means of oxidatively regenerating NAD to support glycolysis, bypassing the fermentative route to ethanol.

The effects of expression of sense and antisense hemoglobin on energy charge are reminiscent of hypoxic acclimation of plant tissues, for example, maize root tips, which develop a tolerance to short term anoxia if they have been acclimated by exposure to hypoxic conditions (Johnson et al, 1989, *Plant Physiol* 91:837-841). Specifically, acclimation is accompanied by increased energy charge (Hole et al, 1992, *Plant Physiol* 99:213-218) resulting from a sustained glycolytic rate compared to non-acclimated root tips (Xia and Saglio, 1992, *Plant Physiol* 100:40-46; Xia and Roberts, 1996, *Plant Physiol* 111:227-233). Similarly, winter cereals show increased survival to hypoxia caused by ice encasement if they have been acclimated by exposure to hypoxic conditions (Andrews and Pomeroy, 1983, *Can J Bot* 61:142-147). Acclimated plants maintain higher levels of adenylates and ATP during ice encasement, as a result of accelerated rates of glycolysis, than non-acclimated plants (Andrews and Pomeroy, 1989, *Plant Physiol* 91:1063-1068). Maximum induction of barley hemoglobin message occurs within 12 hours exposure to hypoxic conditions (Taylor et al, 1994), which is well within the time interval used for acclimation in the above examples. Furthermore, it has been shown that the expression of hemoglobin is not directly influenced by oxygen usage or availability but it is influenced by the availability of ATP in the tissue (Nie and Hill, 1997). This suggests that the increased survival of plants to anoxia as a result of hypoxic acclimation is a consequence of hemoglobin gene expression induced by declining ATP levels during acclimation.

From an evolutionary standpoint, it has been suggested that nonsymbiotic hemoglobins represent one of the more ancient forms of plant hemoglobins (Andersson et al, 1996). Evidence presented here adds credence to this idea. Since early life on earth existed in oxygen-poor environments, the presence of a hemoglobin capable of utilizing oxygen at low oxygen tensions would have provided an evolutionary advantage to an organism. Oxygen produced during photosynthesis and retained as oxyhemoglobin would provide a source of

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oxygen to oxidize NADH, maintaining a high glycolytic flux during darkness to provide ATP for cell growth and development.

The high oxygen avidity of hemoglobin (Arredondo-Peter et al, 1997; Duff et al, 1997; Trevaskis et al, 1997) argues against hemoglobin functioning to facilitate diffusion of oxygen. Because the hemoglobin will be induced intracellularly in a highly reductive environment with low energy charge it is possible that hemoglobin functions as an electron transport protein similar to cytochrome c. Further work is now being carried out to more closely examine the potential effect of oxygen limitation and hemoglobin expression during germination.

The function of this enigmatic protein is still far from certain. We have observed hemoglobin gene expression (or increases in hemoglobin expression) unequivocally in at least 4 cases: (1) in intact whole seeds during germination; (2) in excised embryos and embryo-less half seeds imbibed in water; (3) in aleurone layers which have been stressed by a low oxygen environment or respiratory inhibitors (Nie and Hill, 1997); and (4) in barley roots after flooding (Taylor et al, 1994). In every situation, it is likely that the ATP requirement of the cell exceeds the ATP supply either because of low oxygen supply (such as is the case of the flooded plants or stressed seed tissue) or due to high metabolic rates (such as likely to be the case during germination). Hemoglobin expression seems to be both a normal event during seed germination as well as an adaptation of plants to low oxygen environments.

As discussed above, the results obtained from expression of Hb in bacterial cells are reminiscent of maize suspension cells where it was hypothesized that Hb might be involved in maintaining the level of ATP through the involvement of a pathway other than oxidative phosphorylation. It seems reasonable to conclude that given the similarity of results that a similar mechanism might be occurring in *P. aeruginosa* but not *E. coli*. As discussed above, this is likely due to the fact that *E. coli* adapts readily to grow under conditions of limited oxygen, whereas *P. aeruginosa* is an obligate aerobe and does not normally grow under conditions of limited oxygen. However, the fact that this phenomenon is seen in organisms as diverse as plants and aerobic bacteria further suggests that whatever the function of the nonsymbiotic plant hemoglobin is, it may be widely

represented in nature and may have evolved from a very ancient and fundamental form of oxidative metabolism which evolved before mitochondrial oxidative phosphorylation. This final conclusion is suggested by the fact that Hb can bind oxygen at levels far lower than most other oxygen binding proteins (especially cytochrome C and the alternative oxidase) and may have evolved when oxygen levels in the atmosphere were much lower.

As will be apparent to one knowledgeable in the art, for expressing Hb in a variety of host organisms, expression vectors may be constructed containing Hb linked to a host-specific promoter. Furthermore, the expression vector may contain a selectable marker functional in the specific host for selecting transformants. In this manner, a variety of expression vectors may be constructed for use in a variety of host organisms. Transgenic or recombinant organisms containing these vectors will have increased tolerance to hypoxic conditions, lower levels of fermentation products and increased oxygen uptake. More specifically, plants containing the Hb expression vector described above engineered for expression in a given plant will have improved agronomic properties, such as, for example, germination, seedling vigour, reduced cellular levels of fermentation products, increased oxygen uptake, and increased tolerance to hypoxic conditions.

Furthermore, given that the effect of nonsymbiotic hemoglobin on cell energy status is seen in both bacteria and plants, it seems likely that this phenomenon is universal. This would in turn mean that nonsymbiotic hemoglobins have potential applications in a number of medical procedures. For example, skin cells from burn victims are frequently cultured for transplantation back to the burn victim. Given that oxygen supply is a limiting factor for growth and survival of the transplanted skin grafts, skin cells transfected with nonsymbiotic hemoglobin may possess improved growth and survival. Similarly, oxygen supply is also a limiting factor in other medical procedures, for example, organ transplants. That is, it is likely that organs possessing nonsymbiotic hemoglobins may have enhanced survival following transplant.

As is apparent to one knowledgeable in the art, other oxygen binding proteins displaying a low dissociation constant for oxygen may be used in place of Hb in the above-described expression vectors.

24

Furthermore, as discussed above, the expression of hemoglobin occurs during seedling germination. As such, expression of hemoglobin can be used as a marker for germination. In addition, as discussed above, hemoglobin expression is clearly related to seedling vigour. As such, levels of hemoglobin expression at the time of germination can be used for selecting seeds for breeding.

Since various modifications can be made in our invention as herein above described, and many apparently widely different embodiments of same made within the spirit and scope of the claims without departure from such spirit and scope, it is intended that all matter contained in the accompanying specification shall be interpreted as illustrative only and not in a limiting sense.

25

Table 1. Energy charge and total adenylates in maize cells before and after exposure to a nitrogen atmosphere for 12 hours. Results are expressed as nmol per g fresh weight. Maximum SE (n = 3) was 5%.

| Cell Line | Energy Charge | | Total adenylates (nmol per g fresh weight) | |
|-----------------|---------------|----------|---|----------|
| | Air | Nitrogen | Air | Nitrogen |
| HB ⁺ | 0.93 | 0.93 | 96 | 92 |
| Wild | 0.94 | 0.93 | 94 | 61 |
| HB ⁻ | 0.91 | 0.73 | 99 | 59 |

Table 2 – A₆₀₀ measurements of transformed and untransformed *E. coli* and *P. aeruginosa* cells grown aerobically and anaerobically. Measurements are the averages of two separate determinations which did not vary by more than 15%.

| | <i>E. coli</i> | | <i>P. aeruginosa</i> | |
|---|----------------|-------|----------------------|-------|
| | Wild type | +Hb | Wild type | +Hb |
| 3 hr O ₂ | 0.044 | 0.040 | 0.098 | 0.059 |
| 9 hr O ₂ | 0.147 | 0.110 | 1.392 | 1.074 |
| 3 hr O ₂ + 6 hr N ₂ | 0.144 | 0.102 | 0.141 | 0.074 |

Table 3 – ATP measurements of transformed and untransformed *E. coli* and *P. aeruginosa* cells grown aerobically and anaerobically. Measurements are the results of duplicate assays from 3 separate experiments. Standard error was in all cases no greater than 10%.

| | <i>E. coli</i> | | <i>P. aeruginosa</i> | |
|---|----------------|-------|----------------------|-------|
| | Wild type | +Hb | Wild type | +Hb |
| 9 hr O ₂ | 0.019 | 0.019 | 0.019 | 0.025 |
| 3 hr O ₂ + 6 hr N ₂ | 0.018 | 0.019 | 0.011 | 0.018 |

CLAIMS

1. A recombinant expression system capable, when transformed into an organism, of expressing a gene encoding a nonsymbiotic hemoglobin, which system comprises a nucleotide sequence encoding said nonsymbiotic hemoglobin operably linked to control sequences effective in said organism.
2. The system according to claim 1 wherein the control sequences include a strong constitutive promoter.
3. The system according to claim 1 wherein the nonsymbiotic hemoglobin is barley hemoglobin.
4. The system according to claim 1 wherein the organism is a plant.
5. The system according to claim 4 wherein the plant is maize.
6. The system according to claim 5 wherein the promoter is maize ubiquitin promoter.
7. The system according to claim 1 wherein the organism is a bacteria.
8. The system according to claim 7 wherein the bacteria is an obligate aerobe.
9. The system according to claim 7 wherein the bacteria is *P. aeruginosa*.
10. Cells transformed with the expression system according to any one of claims 1 to 9.
11. A transgenic organism whose genome has been modified to contain the expression system according to any one of claims 1 to 9.
12. A method of increasing tolerance to hypoxic conditions comprising:
 - providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and
 - placing the organism under hypoxic conditions,
 - wherein the oxygen-binding protein acts to maintain cellular energy status during the hypoxic conditions by making oxygen available for cellular metabolism at low oxygen tension.

13. A method of lowering the level of fermentation products in an organism comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

reducing the level of fermentation products in the cells of the organism by maintaining cell energy status such that fermentation is bypassed.

14. A method of maintaining cellular metabolism under hypoxic conditions comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

placing the organism under hypoxic conditions,

wherein the oxygen-binding protein acts to maintain cellular metabolism status by providing oxygen for cellular metabolism.

15. A method of increasing oxygen uptake of an organism comprising:

providing an organism having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

exposing the organism to an oxygen-containing environment,

wherein the increased cellular levels of the oxygen-binding protein results in increased oxygen uptake.

16. A method of improving the agronomic properties of a plant comprising:

providing a plant having increased cellular levels of an oxygen-binding protein having a low dissociation constant for oxygen; and

growing the plant.

17. The method according to claim 16 wherein the improved agronomic properties include germination.

18. The method according to claim 16 wherein the improved agronomic properties include seedling vigour.

19. The method according to claim 16 wherein the improved agronomic properties include reduced cellular levels of fermentation products.

20. The method according to claim 16 wherein the improved

agronomic properties include increased oxygen uptake.

21. The method according to claim 16 wherein the improved agronomic properties include increased tolerance to hypoxic conditions.

22. A method of performing skin grafts comprising:

isolating skin cells from a patient;

transflecting the skin cells with an expression system comprising a nucleotide sequence encoding an oxygen binding protein having a low dissociation constant for oxygen operably linked to control sequences effective in skin cells;

culturing the skin cells such that the oxygen binding protein is expressed; and

grafting the skin cells onto a region of skin tissue attached to the patient.

23. A method of transplanting an organ from a donor to a recipient comprising:

providing an organ for transplant;

infusing the organ with an oxygen binding protein having a low dissociation constant for oxygen, thereby improving oxygen supply to the organ; and

transplanting the organ into the recipient.

24. The method according to any one of claims 12 to 23 wherein the oxygen binding protein having a low dissociation constant for oxygen is a nonsymbiotic hemoglobin.

25. The method according to claim 24 wherein the nonsymbiotic hemoglobin is barley hemoglobin.

26. A method of selecting seeds for breeding to produce seed lines having desirable characteristics comprising:

providing a representative seed of a given seed line;

growing the seed such that the seed germinates;

isolating an extract from the seed;

measuring levels of hemoglobin expression within the extract; and

selecting or rejecting the seed for further breeding based on the hemoglobin levels.

29

27. A method of determining if a seed is germinating comprising:

providing a seed suspected of germinating;

isolating an extract from the seed; and

measuring levels of hemoglobin expression within the extract,

wherein high levels of hemoglobin expression indicate that the seed is germinating.

A. pAS1 (Sense)**B. pAS2 (Anti-sense)****FIG. 1**

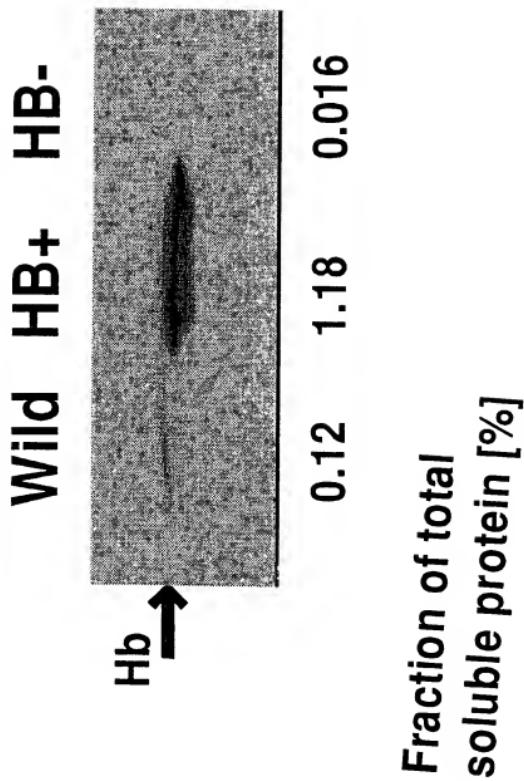


FIG. 2

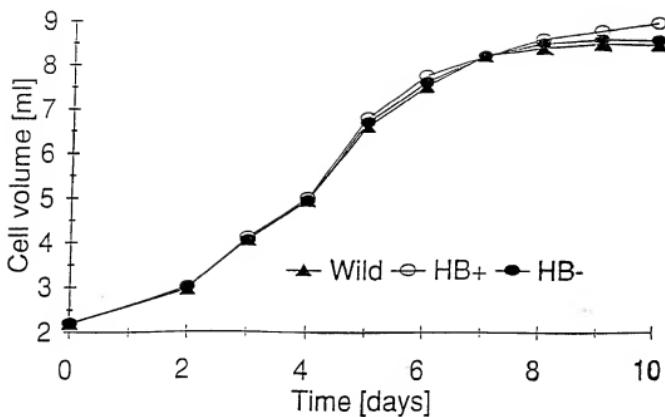


FIG. 3

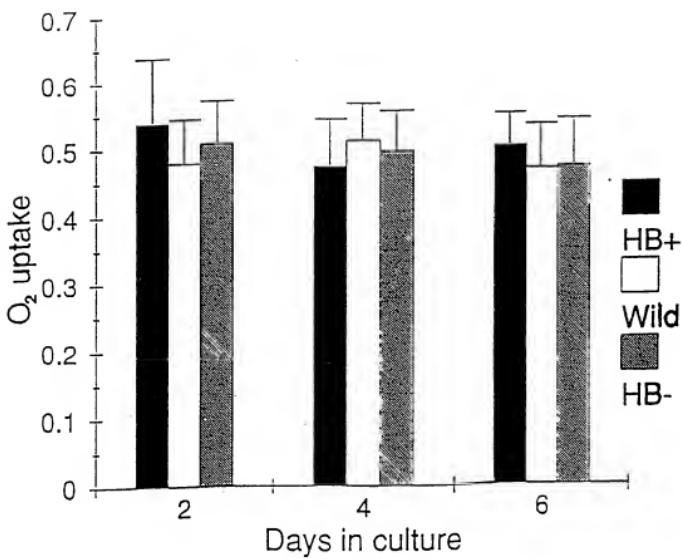


FIG. 4

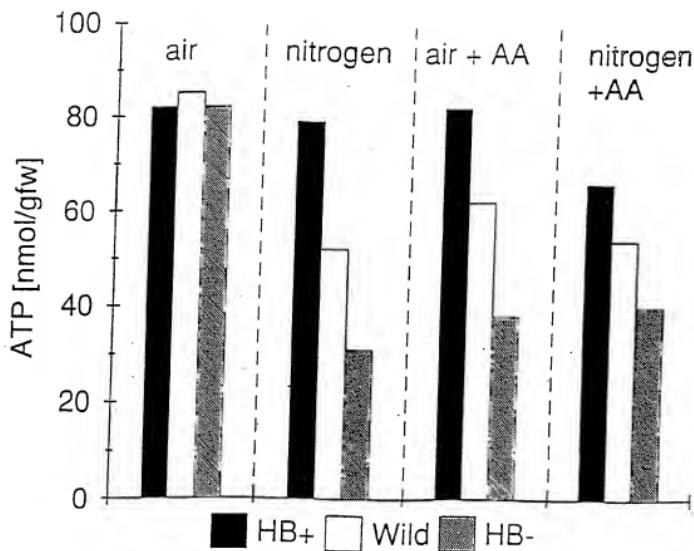


FIG. 5

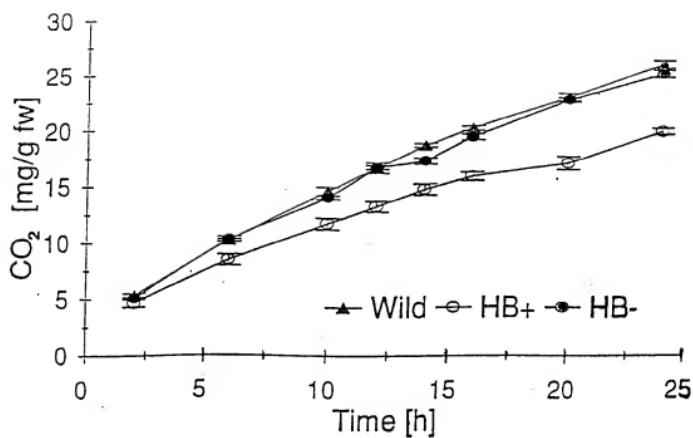


FIG. 6

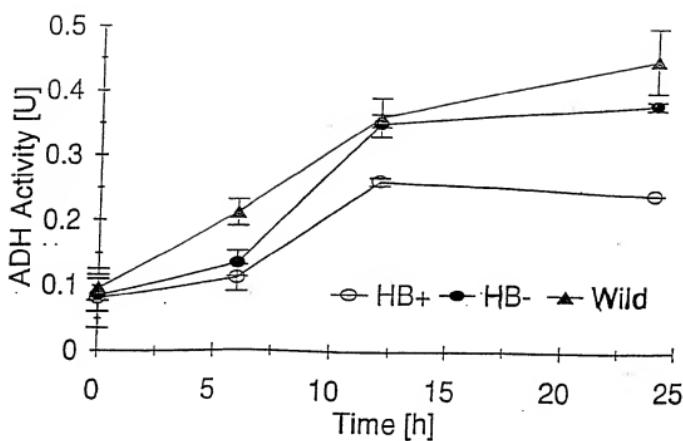


FIG. 7

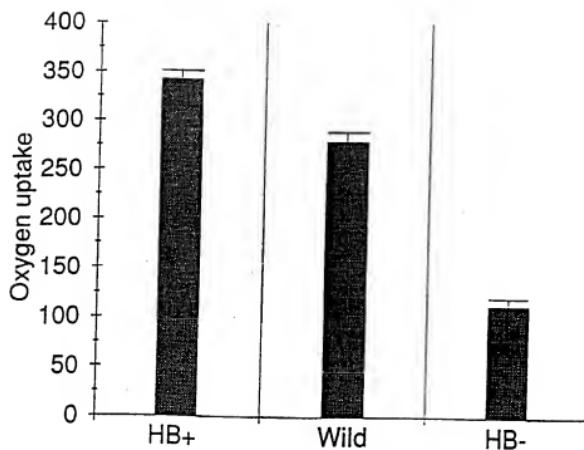


FIG. 8

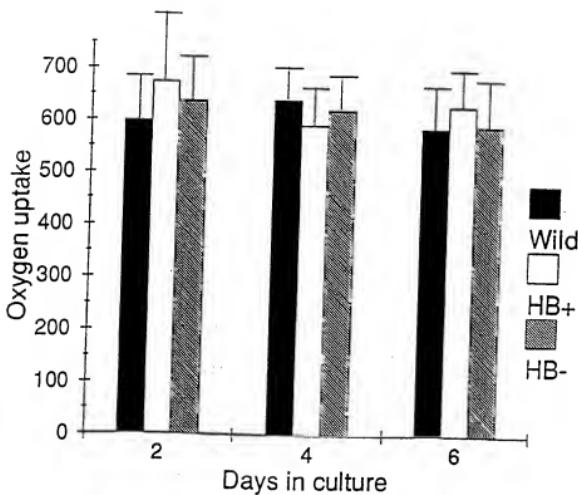


FIG. 9

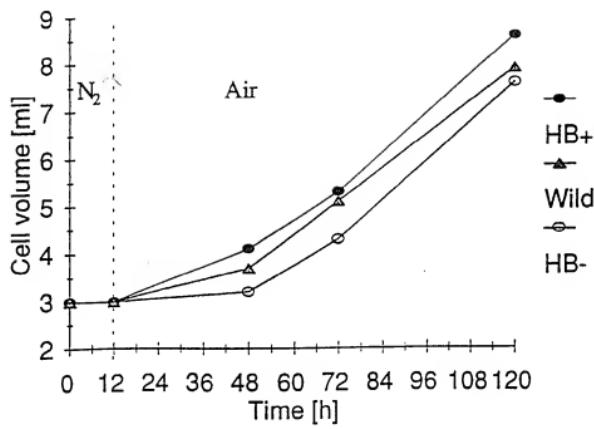


FIG. 10

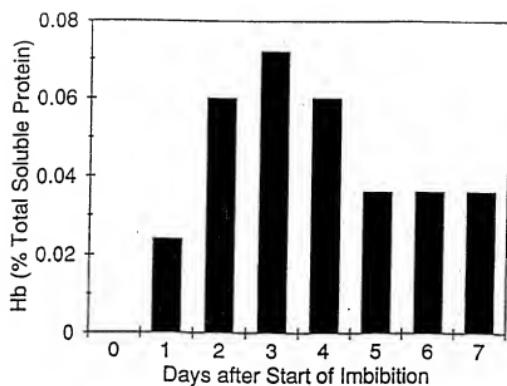


FIG. 11

Lane 1 2

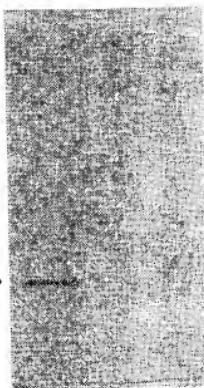


FIG. 12

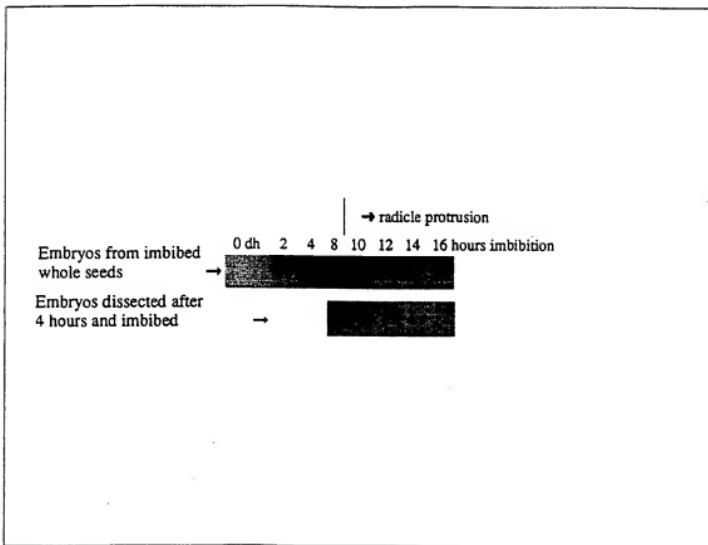
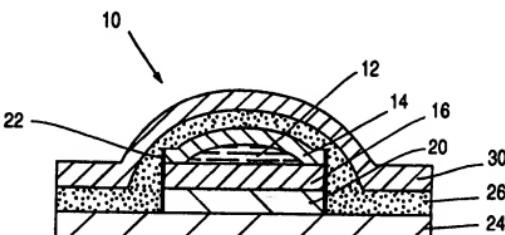


FIG. 13



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
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(54) Title: **TRANSDERMAL DELIVERY SYSTEM WITH ADHESIVE OVERLAY AND PEEL SEAL DISC**

(57) Abstract

A device (10) for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of an adhesive overlay (26), a backing layer (14) underlying the central portion of the adhesive overlay, an active agent-permeable membrane (16), the backing layer and membrane defining a reservoir (12) that contains a formulation of the active agent, a peel seal disc (20) underlying the active agent-permeable membrane, a heat seal (22) about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer and a removable release liner (24) underlying the exposed overlay and peel seal disc. The adhesive layer is above and peripheral to the path of the active agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. The peel seal disc protects against release of the active agent-containing reservoir and the release liner protects the adhesive from exposure to the environment prior to use.

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5 TRANSDERMAL DELIVERY SYSTEM WITH ADHESIVE OVERLAY AND
 PEEL SEAL DISC

Technical Field

10 This invention is in the field of transdermal and transmucosal administration of active agents (drugs). More particularly, it relates to a device for achieving such administration that has an active agent-containing reservoir and an adhesive layer for affixing the device
15 to the skin or mucosa. The adhesive layer is above and peripheral to the path of the active agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. A peel seal disc protects against release of the
20 active agent-containing reservoir and a release liner protects the adhesive from exposure to the environment prior to use.

Background of the Invention

25 Devices for administering drugs through the skin or mucosa have been described in many patents. These devices are usually of two types: matrix systems and liquid reservoir systems. Both systems are laminated composites that have, from basal to top, a peelable
30 release liner, a pressure sensitive adhesive layer for attaching the device to the skin, a drug-containing layer, and a drug impermeable backing layer. In the matrix type system, the drug is dispersed within a solid or semi-solid carrier. In the reservoir type system, the
35 drug, typically in liquid solution, is contained within a

walled container, usually formed between the backing layer and a drug-permeable membrane layer.

A number of reservoir type systems have been described. U.S. Patent No. 4,710,191 to Kwiatek et al.

5 involves a reservoir type device that includes a peelable heat sealed release liner layer underlying the reservoir and the portion of the backing layer that extends beyond the reservoir. In a second embodiment, a microporous membrane is included between the backing layer and the
10 reservoir.

U.S. Patent No. 4,829,224 to Chang et al. describes a device with a reservoir that is defined by a backing layer and a drug-permeable membrane layer. A ring-shaped layer made of an adhesive is peripheral to
15 the reservoir. A peelable liner layer underlies the membrane. A second peelable layer, the release liner, underlies the entire assembly. A first heat seal connects the backing layer and the membrane and surrounds the reservoir. A second heat seal concentric about the
20 first heat seal connects the backing layer and the release liner. The second heat seal is broken when the release liner is removed. The device may include an inner liner that underlies the membrane and portions of the backing layer. This inner liner is removed following
25 removal of the release liner so that the membrane is exposed.

U.S. Patent No. 4,983,395 to Chang et al. relates to another device with a backing layer and a membrane layer that define a reservoir. A peelable inner liner underlies the reservoir and portions of the backing and membrane layers outside the periphery of the reservoir. An adhesive layer underlies the inner liner and remaining portions of the backing and membrane layers. A peelable release liner underlies the adhesive
35 layer. A first heat seal connects the backing and

membrane layers on the periphery of the reservoir. A second heat seal underlies the first heat seal and connects the membrane and the inner liner. In use, the release liner and inner liner are peeled away to expose
5 the undersurfaces of the membrane and adhesive layers prior to placement of the device onto the skin or mucosa.

The present invention is an improved device for transdermal or transmucosal drug delivery in which an occlusive peel seal layer underlies the drug containing
10 reservoir and serves to prevent release of the drug prior to use. A release liner overlays the peel seal layer and exposed portions of the adhesive overlay. In this way, the release liner need not be occlusive and thus may be chosen from a wide variety of materials that can be
15 breathable, and can have a stretchable, elastic quality. Such devices are cost efficient and easy to manufacture.

In addition, in the devices of this invention only the central portion of the device in line with the
20 reservoir is occlusive, leaving the portion of the device peripheral to the central portion nonocclusive or breathable.

Disclosure of the Invention

25 The invention is a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of:
30 (a) an adhesive overlay having a central portion and a peripheral portion;
(b) a backing layer underlying the central portion of the adhesive overlay;
(c) an active agent-permeable membrane underlying the backing layer, the backing layer and membrane defining

- (d) a reservoir therebetween that contains an active agent;
- (e) a peel seal disc underlying the active agent-permeable membrane;
- 5 (f) a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer; and
- (g) a removable release liner underlying the peripheral portion of the adhesive overlay and the peel seal disc.
- 10

Brief Description of the Drawings

Figure 1 is an enlarged sectional view of one embodiment of the device of the invention.

15 Figures 2A and 2B are enlarged top views of further embodiments of the device of the invention.

Figure 3 is an enlarged sectional view of another embodiment of the invention.

20 Figures 4 is an enlarged view of the embodiment of Figure 3 showing the embodiment disassembled.

Figure 5 is a graph showing a comparison of calculated and experimental cumulative release kinetics of keterolac tromethamine based on the tests described in Example 23.

25

Modes for Carrying Out the Invention

Figure 1 shows a device, generally designated 10 that is designed to administer a formulation of an active agent to the skin or mucosa. The device 10 is a 30 laminated composite. A drug reservoir 12 is formed between an upper backing layer 14 and an underlying drug-permeable membrane layer 16. A peel seal disc 20 is placed beneath the membrane layer 16. The three layers, the backing layer 14, the membrane layer 16 and the peel seal disc 20 are heat sealed together at their periphery

35

22, the membrane layer 16 being heat sealed to the
backing layer 14 and the peel seal disc 20 being heat
sealed to the membrane layer 16. A release liner 24 is
placed underneath the peel seal disc 20 and extends
5 beyond the peripheral heat seal 22. An adhesive layer 26
overlies the backing layer 14 and the portions of the
release liner 24 that extend beyond the peripheral heat
seal 22. An uppermost overlay layer 30 overlies the
10 adhesive layer 26. In this embodiment the release liner
is removed together with the peel seal disc prior to
placing the device on the skin.

Figures 2A and 2B show further embodiments of
the device where a tab 32 extends past the heat seal 22
and allows for easy removal of the peel seal disc 20.
15 The tab 32 may extend a short distance past the heat seal
22 as shown in Figure 2A or may extend to the periphery
34 of the device 10 as shown in Figure 2B.

Figures 3 and 4 depict another embodiment,
generally designated 40, of the laminated composite of
20 the invention. The device of Figures 3 and 4 differs
from that of Figure 1 by the presence of a second
adhesive layer that underlies the peel seal disc and the
peripheral portion of the first adhesive layer. More
specifically, device 40 consists of (from top surface to
25 basal surface) a nonocclusive overlay layer 42, a first
adhesive layer 44, a backing layer 46, a drug reservoir
48 comprising a solution or gelled formulation of a
transdermally administrable drug; a drug-permeable
membrane layer 50; a peel seal disc 52, a second adhesive
30 layer 54, and a release liner layer 56. As shown, the
backing film, reservoir, membrane, and peel seal disc are
"in-line" and have smaller areas than the overlay, the
two adhesive layers and the release liner. The latter
extend peripherally beyond the entire periphery of the
35

former. The backing film, membrane, and peel seal disc are heat sealed at 60 about their entire peripheries.

In use the release liner layer, the portion of the second adhesive layer that underlies the peel seal disc, and the peel seal disc are separated from the rest of the assembly. The separated or disassembled configuration is shown in Figure 4. In this regard, the relative bond strengths between the release layer and the second adhesive layer, the second adhesive layer and the first adhesive layer and peel seal disc, and the peel seal disc and membrane are such that when the release liner is removed, the central portion of the second adhesive layer and the peel seal disc are removed with it. The removed release layer subassembly is discarded. The subassembly containing the drug reservoir is placed on the skin, with the peripheral ring of the second adhesive layer being the means by which that subassembly is affixed to the skin or mucosa. When so placed on the skin the drug reservoir is in diffusional communication with the skin via the membrane. In other words, drug is free to diffuse through the membrane to the skin.

The backing layer 14 or 46 of the device may be composed of a single film or a plurality of films. In any event, its inner surface must be capable of being heat sealed to the membrane layer 16 or 50. One or more of the films that constitute the backing layer will be impermeable to components of the drug formulation contained in the reservoir. Examples of materials used as backing layers in transdermal delivery devices that may find use in the present invention are polyethylene, polypropylene, polyvinylchloride, polyethylene terephthalate, ethylene-vinyl acetate copolymers and combinations thereof. The layer may include one or more metal layers and/or one or more fibrous layers. Preferably the backing layer is occlusive.

The term "active agent" or "drug" as used to describe the principal active ingredient of the device intends a biologically active compound or mixture of compounds that has a therapeutic, prophylactic or other beneficial pharmacological and/or physiological effect on the wearer of the device. Examples of types of drugs that may be used in the inventive device are antiinflammatory drugs, analgesics, antiarthritic drugs, antispasmodics, antidepressants, antipsychotic drugs, tranquilizers, antianxiety drugs, narcotic antagonists, antiparkinsonism agents, cholinergic agonists, anticancer drugs, immunosuppression agents, antiviral agents, antibiotic agents, appetite suppressants, antiemetics, anticholinergics, antihistamines, antimigraine agents, coronary, cerebral or peripheral vasodilators, hormonal agents, contraceptive agents, antithrombotic agents, diuretics, antihypertensive agents, cardiovascular drugs, and the like. The appropriate drugs of such types are capable of permeating through the skin either inherently or by virtue of treatment of the skin with a percutaneous absorption enhancer. Because the size of the device is limited for patient acceptance reasons, the preferred drugs are those that are effective at low concentration in the blood stream. Examples of specific drugs are steroids such as estradiol, progesterone, norgestrel, levonorgestrel, norethindrone, medroxyprogesterone acetate, 3-ketodesogestrel, testosterone and their esters, nitro-compounds such as nitroglycerine and isosorbide nitrates, nicotine, chlorpheniramine, terfenadine, triprolidine, hydrocortisone, oxicam derivatives such as piroxicam, ketoprofen, mucopolysaccharidases such as thiomucase, buprenorphine, fentanyl, naloxone, codeine, dihydroergotamine, pizotiline, salbutamol, terbutaline, prostaglandins such as misoprostol and enprostil, omeprazole, imipramine,

benzamides such as metoclopramide, scopolamine, peptides such as growth releasing factor and somatostatin, clonidine, dihydropyridines such as nifedipine, verapamil, ephedrine, pindolol, metoprolol,
5 spironolactone, nicardipine hydrochloride, calcitriol, thiazides such as hydrochlorothiazide, flunarizine, sydononimines such as molsidomine, sulfated polysaccharides such as heparin fractions and the salts of such compounds with pharmaceutically acceptable acids
10 or bases.

In addition to the drugs described above, depending on the permeability of the skin to the drug or drugs, the reservoir may also contain a percutaneous absorption enhancer that increases the permeability of the skin to the drug or drugs and is coadministered to the skin. Examples of percutaneous absorption enhancers are those referred to in U.S. Patents Nos. 3,989,816, 4,863,970, 4,316,893, 4,405,616, 4,060,084 and 4,379,454 and *J. Pharm Sci* (1975) 64:901-924. The formulation contained in the reservoir may further include solvents, gelling agents, stabilizers, antiirritants, and other additives.
15
20

The membrane layer 16 or 50 is permeable to the drug. It may be a "dense" membrane made of material that is inherently permeable to the components of the reservoir that are to be administered to the skin or mucosa or it may be made of a microporous membrane whose pores are filled with a drug-permeable material including the drug reservoir formulation itself which may include enhancers where desired. In the case of dense membranes, the components dissolve in the material and diffuse through the material to the skin. In the case of microporous materials, the components diffuse through the pores to the skin. The membrane may or may not be a rate-controlling element depending upon the particular
25
30
35

drug involved, the permeability of the skin to the drug, and the rate of delivery required to provide therapy.

Examples of materials for making dense membranes are given in U.S. Patents Nos. 3,598,122 and 4,650,484.

- 5 Examples of materials for making microporous membranes are provided in U.S. Patents Nos. 3,797,494 and 4,031,894.

The adhesive layers 26, 44 and 54 are composed of pressure sensitive surgical adhesive such as those 10 that are commonly used to affix transdermal drug delivery devices, bandages or other dressings to the skin. Examples of such adhesives are polyisobutene (PIB), natural rubber adhesives, acrylate and methacrylate adhesives and silicone adhesives. Breathable, 15 nonocclusive adhesives such as the acrylate/methacrylate and silicone adhesives are preferred.

The peel seal disc 20 or 52 may be composed of a single layer or a multiplicity of layers. The disc should be (1) impermeable to the components of the drug 20 reservoir formulation that diffuse through the membrane, (2) heat-sealable to the membrane layer, and (3) inherently strippable or rendered so by techniques such as silicone or fluorocarbon treatment or surface treatment with a seal incompatible layer. Suitable peel 25 seal discs include those made from Bertek 4418 Peelable Seal, Total Healthcare Packaging TPC-0812 or TPC-0760 and UCB Medical Industries LR 4/25.

The release liner 24 or 56 may similarly be composed of a single layer or a multiplicity of layers. 30 Unlike the peel seal disc, however, the release liner need not be impermeable to the components of the drug formulation in that the peel seal disc protects against release of the components from the reservoir. Therefore, suitable release liners may be made from materials such 35 as polyester, low density polyethylene (LDPE), high

- density polyethylene (HDPE), polypropylene, polystyrene, polyamide, nylon, polyvinyl chloride and specialty papers, and include Akrosil Biorelease liners, Scotchpak 1022 release liners, Adhesives Research AR5MS, Custom 5 Coating and Laminating 7000 on HDPE or 6020 on polyethylene terephthalate (PET).

The overlay layer 30 or 42 overlies the adhesive layer 26 or 44 and may be made from materials such as polyolefin, polyurethane, nylon, polyester, 10 vinyl, acetate taffeta or other elastomeric woven or nonwoven fabrics or films. Preferably the overlay material is nonocclusive (breathable).

The respective components of the device may be formulated and assembled using procedures that are known 15 in the drug formulation, transdermal device and laminating arts. The shape of the device is not critical, and devices of preformed shapes may be assembled directly or punched, cut or otherwise formed from large sheets of laminated composite.

20 The following examples further illustrate the invention. These examples are not intended to limit the invention in any manner.

EXAMPLES

25

Example 1

A medical grade, pressure sensitive acrylic adhesive, MA-31 (Adhesives Research, Glen Rock, PA) was coated onto a low peel strength, siliconized release 30 liner using a 10 mil gap casting knife. The adhesive solvent was evaporated at 80°C for 15 min. to provide a final dry adhesive thickness of .002 inches. Volara Foam 15EO (Voltek, Division of Sekisui America Corp, Lawrence MA) the overlay material, was then laminated onto the dry 35 adhesive film forming the overlay adhesive laminate.

This overlay adhesive laminate was used at a later stage of the fabrication process as described below.

A gelled testosterone formulation was prepared by dissolving 15 mg/ml testosterone in a solution containing 50.0/15.0/30.0/2.5/2.5% (v/v) of ethanol/water/glycerin/glycerol monooleate/methyl laurate. The resulting solution was gelled with 3.0% (w/v) of Carbopol 1342. The pH of the resulting gel was adjusted to 4.5 to 5.0 by addition of 2N sodium hydroxide.

The overlay patch with peel seal disc was fabricated as follows: A CoTran 9711 microporous membrane (3M, St. Paul, MN) was placed over an LR4/25 peel seal disc film (UCB Medical Industries, Bloomfield, CT). 0.9 g of the testosterone formulation was dispensed onto the microporous membrane. The backing film (Scotchkpak 1012, 3M, St. Paul MN) was predimpled to provide a flat, circular cup with a volume of approximately 1.0 cc. This predimpled backing film was placed over the gel such that the dimple in the backing film was centered on the mound of gel. The backing film was then heat sealed to the microporous membrane/peel seal film laminate at 370°F with a pressure of 30 psi and a 0.4 sec. dwell time. A circular heat seal die with a interior diameter of 1.22 inches and a heat seal width of 0.03 inch was used. A gel containing reservoir with an active surface of 7.5cm² was formed. This heat sealing step simultaneously creates a permanent reservoir seal between the microporous membrane and the backing film and a peelable seal between the microporous membrane and the peel seal disc film. This intermediate drug containing reservoir system, with a diameter of 3.5 cm and a total surface area of 9.6 cm², was then diecut from the heat sealed laminate.

The above reservoir system was laminated to the previously prepared overlay adhesive laminate by removing the protective release liner from the overlay adhesive, placing the diecut reservoir system onto the adhesive
5 with the backing film contacting the adhesive, relaminating the release liner over the exposed adhesive and finally diecutting the actual overlay adhesive transdermal delivery system from this laminate. The cutting die used for this operation has a diameter of 5.5
10 cm thereby diecutting a transdermal delivery system with a final surface area of 23.8 cm², which includes the peripheral adhesive area. The activation of this system before application to the skin proceeds in a two step process: the release liner covering the adhesive is
15 removed first, followed by the removal of the peelable disc film protecting the reservoir.

Example 2

Transdermal systems with an adhesive overlay
20 were made using the procedure described in Example 1, except that the adhesive used was a medical grade acrylic adhesive, Gelva 737 (Monsanto, St. Louis, MO).

Example 3

Transdermal systems with an adhesive overlay
25 were made using the procedure described in Example 1, except that the adhesive used was a medical grade PIB adhesive MA-24 (Adhesives Research, Glen Rock, PA).

Example 4

Transdermal systems with an adhesive overlay
were made using the procedure described in Example 1,
except that a silicone based adhesive, BIO PSA X7-2920
(Dow Corning, Midland, MI) was used. The release liner

used was the Akrosil BioRelease liner (Menasha, WI) which is compatible with the above silicone adhesive.

Example 5

5 Transdermal systems with an adhesive overlay were made using the procedure described in Example 1, except that the adhesive used was an (acrylic copolymer) adhesive, TSR Adhesive (Sekisui Chemical Company, Osaka, Japan).

10

Example 6

Transdermal systems with an adhesive overlay were made using the same procedure as described in Example 1, except that the peel seal disc film was a
15 multilaminate PET/LDPE/Foil/Primacor 3440/LLDPE/Heat Seal Film flexible packaging material, TPC-0812 (Tolas Healthcare Packaging, Feasterville, PA).

20

Example 7

Transdermal systems with an adhesive overlay were made using the same procedure as described in Example 1, except that the peel seal disc film was a
Peelable Foil Film material, a multilaminate of
25 PET/adhesive/Foil/adhesive/LLDPE/Heat Seal Film (Technipaq, Crystal Lake, IL).

30

Example 8

Transdermal systems with an adhesive overlay were made using the procedure described in Example 1,
except that the overlay material was an embossed
polyurethane elastomeric film.

35

Example 9

Transdermal systems with an adhesive overlay were made using the procedure described in Example 1,

except that the overlay material was a 100% nonwoven polyester fabric.

Example 10

5 Transdermal systems with an adhesive overlay were made using the procedure described in Example 1, except that the overlay material was a spunbonded nylon film.

10

Example 11

Transdermal systems with an adhesive overlay were made using the procedure described in Example 1, except that the overlay material was a woven acetate taffeta film.

15

Example 12

A 4 in. x 4 in. commercially available wound dressing, Mitraflex[®] Plus, distributed by Calgon Vestal Laboratories, was used as the adhesive overlay.

20

A gelled ketorolac tromethamine formulation was prepared by dissolving 60 mg/ml ketorolac tromethamine in a solution containing 55.28/33.17/10.0/1.55% (v/v) of isopropyl alcohol/water/glycerin/isopropyl myristate. The resulting solution was gelled with 5.0% (w/v) of 25 Natrosol[®] Plus 330 CS and the pH of the resulting gel was adjusted to 5.1 ± (0.2) by addition of 2N HCl. The gel also contained 0.1% (w/v) butylated hydroxy toluene as an antioxidant.

30

The overlay patch with peel seal disc was fabricated as follows: A CoTran 9710 microporous membrane was placed over an LR4/25 peel seal disc film. 3.44 g of the ketorolac tromethamine formulation was dispensed onto the microporous membrane. The backing film was predimpled to provide a flat, circular cup.

35

This predimpled backing film was placed over the gel such

that the dimple in the backing film was centered on the mound of gel. The backing film was then heat sealed to the microporous membrane/peel seal film laminate at 370°F with a pressure of 30 psi and a 0.4 sec. dwell time. An 5 oval heat seal die was used to form a gel containing reservoir with an active surface of 30 cm². This heat sealing step simultaneously creates a permanent reservoir seal between the microporous membrane and the backing film and a peelable seal between the microporous membrane 10 and the peel seal disc film. This intermediate drug containing reservoir system, with a total surface area of 42.55 cm², was then diecut from the heat sealed laminate.

The above reservoir systems were laminated to the Mitraflex[®] Plus overlay adhesive laminate by removing 15 the protective release liner from the wound dressing, placing the diecut reservoir system onto the adhesive with the backing film contacting the adhesive and relaminating the release liner over the exposed adhesive to obtain the actual overlay adhesive transdermal 20 delivery system. The activation of this system before application to the skin proceeds in a two step process: the release liner covering the adhesive is removed first, followed by the removal of the peelable disc film protecting the reservoir.

25

Example 13

Transdermal systems with an adhesive overlay were made using the procedure described in Example 12, except that the overlay adhesive laminate used was 30 fabricated as in Example 1. The final surface area of the transdermal delivery system was 63 cm².

Example 14

Transdermal systems with an adhesive overlay 35 were made using the procedure described in Example 13,

except that the adhesive used was a medical grade PIB adhesive, MA-24 (Adhesives Research, Glen Rock, PA).

Example 15

5 Transdermal systems with an adhesive overlay were made using the procedure described in Example 13, except that a silicone based adhesive, BIO PSA X7-2920 (Dow Corning, Midland, MI). The release liner used was the Akrosil BioRelease liner (Menasha, WI) which is
10 compatible with the above silicone adhesive.

Example 16

15 Transdermal systems with an adhesive overlay were made using the procedure described in Example 13, except that the adhesive used was an acrylic copolymer adhesive, TSR Adhesive (Sekisui Chemical Company, Osaka, Japan.

Example 17

20 Transdermal systems with an adhesive overlay were made using the procedure described in Example 13, except that the peel seal disc film was a multilaminate of PET/LDPE/Foil/Primacor 3440/LLDPE/Heat Seal film flexible packaging material, TPC-0812 (Tolas Healthcare
25 Packaging, Feasterville, PA).

Example 18

Transdermal systems with an adhesive overlay were made using the same procedure as described in
30 Example 13, except that the peel seal disc film was a Peelable Foil Film, a multilaminate PET/adhesive/
Foil/adhesive/LLDPE/Heat Seal Film (Technipaq, Crystal Lake, IL).

Example 19

Transdermal systems with an adhesive overlay
were made using the procedure described in Example 13,
5 except that the overlay material was an embossed
polyurethane elastomeric film.

Example 20

Transdermal systems with an adhesive overlay
10 were made using the procedure described in Example 13,
except that the overlay material was a 100% nonwoven
polyester fabric.

Example 21

Transdermal systems with an adhesive overlay
15 were intermediate drug-c made using the procedure
described in Example 13, except that the overlay material
was a spunbonded nylon film.

Example 22

Transdermal systems with an adhesive overlay
20 were made using the procedure described in Example 13,
except that the overlay material was a woven acetate
taffeta film.

25

Example 23

Ketorolac tromethamine gel composition was
prepared as described in Example 12. The gel was
evaluated for in vitro skin flux performance.

In vitro human cadaver skin flux studies were
30 conducted using modified Franz diffusion cells, placed in
a water bath calibrated to maintain the skin surface
temperature at 32°C. The epidermal membrane was
separated from the full thickness human cadaver skin by
the method of Kligman and Christopher (Arch. Dermatol.
35 88, 702-705, 1963). The full thickness skin was exposed

to 60°C heat for 60 seconds. The stratum corneum and epidermis were gently peeled from the dermis. The epidermal membrane was mounted onto a 0.65 cm² surface area diffusion cell with the epidermal side facing the receiver compartment and clamped in place. The receiver compartment was then filled with an aqueous solution containing 0.02% sodium azide as a bacteriostat. The cell was placed in a circulating water bath calibrated to maintain skin surface temperature at 32±1°C and the skin was allowed to hydrate overnight. The subsequent morning, 75µl of the gelled ketorolac formulation was pipetted into a cavity created by placing a Teflon washer over the stratum corneum surface. The cavity was then occluded by clamping an occlusive backing film over the Teflon washer assembly. Throughout the experiment, the receiver compartment, which contained a bar magnet, was continuously stirred by a magnetic stirrer placed under the water bath. At predetermined intervals, the entire contents of the receiver compartment was collected for drug quantitation using the HPLC method in Table I. The receiver compartment was refilled with fresh receptor medium, taking care to eliminate any air bubbles at the skin/solution interface. From the concentration versus time curve, the average steady state flux, the interval flux and cumulative delivery per unit area at 24 hours were calculated. The in vivo delivery was projected by multiplying the in vitro cumulative delivery (µg/cm²/24h) by the patch active area (30 cm²).

Table I
In Vitro Skin Flux
HPLC Method

| | | |
|----|-------------------|--|
| 5 | Column: | Partisphere C-18 (100 mm) |
| | Flow Rate: | 1.0 ml/min |
| 10 | Wavelength: | 314 nm |
| | Injection Volume: | 10 μ l |
| | Retention Time: | 2.0 \pm 0.3 min |
| 15 | Mobile Phase: | 60/40/0.5 (%v/v) Acetonitrile/H ₂ O/Glacial Acetic Acid pH ~ 3.0 |

Overlay patches, prepared as described in
Example 12, were applied to the chest in 24 healthy adult
volunteers (12 male and 12 female) in human clinical
trials. Plasma samples were collected for ketorolac
quantitation at predetermined times. After removal of
the patches in each study, the used patches were
extracted in 100 ml of methanol and analyzed for drug
content using the HPLC method described in Table II. The
difference between the initial and final patch drug
contents gave the amount of ketorolac delivered, based on
patch depletion analysis.

Table II
Patch
Content Assay
HPLC Method

5

10

15

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| | |
|-------------------|--|
| Column: | Zorbax RX-C8 5 μ m, 15 cm x 4.6 mm |
| Flow Rate: | 1.0 ml/min |
| Wavelength: | 314 nm |
| Injection Volume: | 10 μ l |
| Retention Time: | 5.2 \pm 0.2 min |
| Mobile Phase: | 60/40/1 (%v/v) Acetonitrile/H ₂ O/Glacial Acetic Acid pH - 3.0 |

Table III compares the in vivo cumulative delivery, based on patch depletion analysis, with the projected drug delivery for the 30 cm² overlay patch, based on in vitro skin flux results. The projected drug delivery is based upon average in vitro cumulative deliver (μ g/cm²/24h) multiplied by the active area (30 cm²) of the overlay patch. The average in vivo dose was calculated from the difference between initial drug content of the patches and the residual drug content of the patches upon removal. In vivo, the 30 cm² overlay patch delivered (46.4 \pm 17.5) mg/day of ketorolac tromethamine, in very good agreement with the predicted value of 53.7 mg/day. Figure 5 shows that the average in vivo cumulative input kinetics, calculated according to the method of Wagner and Nelson (J. Pham. Sci. 52, 610-611, 1963) is in very good agreement with the in vitro cumulative input kinetics.

Table III

Projected and Observed
In Vivo Performance
of Overlay Patch System

5

10

| Patch System | Projected Dose Ketorolac Tromethamine (mg/day) | In Vivo Dose Ketorolac Tromethamine (mg/day) |
|--|--|---|
| 30 cm ² Overlay Patch | 53.7 | 46.4 ± 17.5 (24 subjects) |

15

Example 24

Transdermal systems with an adhesive overlay were made using the procedure described in Example 1, except that the die used to diecut the liquid reservoir portion of the transdermal delivery system allowed the 20 peel seal disc tab to reach the edge of the release liner, as shown in Figure 2B. This extended tab allows the final system to be activated in a single step process, instead of the two step process described in Example 1. In the single step process, the release liner 25 and the extended tab of the peel seal disc are held between the thumb and index finger of one hand and the backing film in the other. The peel seal disc and release liner are pulled apart from the backing film to activate the system.

30

Example 25

A medical grade pressure sensitive adhesive, MA-31 (Adhesives Research, Glen Rock, PA) was coated onto a high peel strength, siliconized release liner using a 35 10 mil gap casting knife. The adhesive solvent was

evaporated at 80°C for 15 min. to provide a final dry adhesive thickness of .0015 inches. A low strength, in-process, 2 mil high density polyethylene siliconized liner was then laminated onto the dry adhesive film forming the 5 primary adhesive laminate. This primary adhesive laminate, and the overlay adhesive laminate fabricated as described in Example 1, were used at a later stage of the fabrication process as described below.

The intermediate drug containing reservoir was 10 fabricated as an Example 1, except that the weight of the testosterone gel was 1.8g, the active surface was 15 cm², and the total surface area was 18.4 cm². This reservoir system was laminated to the primary adhesive laminate by removing the in-process liner, placing the diecut 15 reservoir centrally onto the adhesive with the peel-seal disc film contacting the adhesive. The protective liner from the overlay adhesive laminate was then removed and the overlay adhesive laminated onto the primary adhesive/reservoir system, such that the overlay adhesive 20 was in contact with the backing film of the intermediate drug-containing reservoir and also the adhesive area of the primary adhesive laminate extending beyond the intermediate drug-containing reservoir. The final overlay adhesive transdermal delivery system was diecut 25 from this laminate structure to give a system with a total surface area of 33 cm².

The activation of this system is carried out in a single step by pulling away the high peel strength liner from the overlay adhesive. The peel-seal disc 30 remains anchored to the liner, thereby exposing the reservoir area.

Example 26

- Transdermal systems with an adhesive overlay were made using the same procedure as described in
5 Example 25, except that the adhesive used in the primary adhesive laminate and the overlay adhesive laminate was a medical grade polyisobutene (PIB) adhesive MA-24 (Adhesives Research, Glen Rock, PA).

10

Example 27

- Transdermal systems with an adhesive overlay were made using the same procedure as described in
Example 25, except that the adhesive used in the primary adhesive laminate and the overlay adhesive laminate was a
15 silicone based adhesive, BIO PSA X7-2920 (Dow Corning, Midland, MI).

- Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in the fields of chemistry, transdermal drug
20 delivery, pharmacology and related fields are intended to be within the scope of the following claims.

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CLAIMS

We claim:

1. A device for administering an active agent
5 to the skin or mucosa of an individual comprising a
laminated composite of:
 - (a) an adhesive overlay having a central portion and a peripheral portion;
 - (b) a backing layer underlying the central portion of the adhesive overlay;
 - 10 (c) an active agent-permeable membrane underlying the backing layer, the backing layer and membrane defining
 - (d) a reservoir therebetween that contains a formulation of the active agent;
 - (e) a peel seal disc underlying the active agent-permeable membrane;
 - (f) a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer; and
 - 20 (g) a removable release liner underlying the peripheral portion of the adhesive overlay and the peel seal disc.
- 25 2. The device of claim 1 wherein the adhesive is made of a material selected from the group consisting of polyisobutenes, natural rubbers, acrylates, methacrylates and silicone.
- 30 3. The device of claim 1 wherein the backing layer comprises at least one impermeable film layer.
4. The device of claim 1 wherein the membrane is selected from the group consisting of microporous membranes and dense membranes.
35

5. The device of claim 1 wherein the peel seal disc is composed of at least one layer.

5 6. The device of claim 1 further comprising
a percutaneous absorption enhancer.

7. The device of claim 1 whenever the formulation further comprises a material selected from the group consisting of a solvent, a gelling agent, 10 stabilizer and an anti-irritant.

8. The device of claim 1 including an adhesive layer that underlies the peripheral portion of the adhesive overlay and the peel seal disc and overlies the 15 release liner.

9. The device of claim 1 wherein the adhesive overlay is nonocclusive and the backing layer is occlusive.

20 10. A device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of:

- (a) an adhesive overlay having a central portion and a peripheral portion;
- (b) a backing layer underlying the central portion of the adhesive overlay;
- (c) an active agent-permeable membrane, the backing layer and membrane defining
- 30 (d) a reservoir therebetween that contains a formulation of the active agent;
- (e) a peel seal disc underlying the active agent-permeable membrane;

(f) a heat seal about the periphery of the
peel seal disc, the active agent-permeable membrane and
the backing layer; and

5 (g) a removable release liner underlying the
peripheral portion of the adhesive overlay and the peel
seal disc; and

(h) a tab on said peel seal disc to aid in
removal of said disc.

10 11. The device of claim 10 including an
adhesive layer that underlies the peripheral portion of
the adhesive overlay and the peel seal disc and overlies
the release liner.

15 12. The device of claim 10 wherein the
adhesive overlay is nonocclusive and the backing layer is
occlusive.

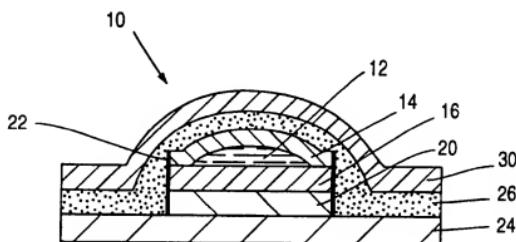
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**FIG. 1**

2/4

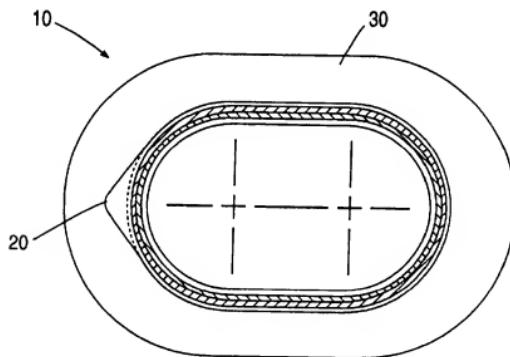


FIG. 2A

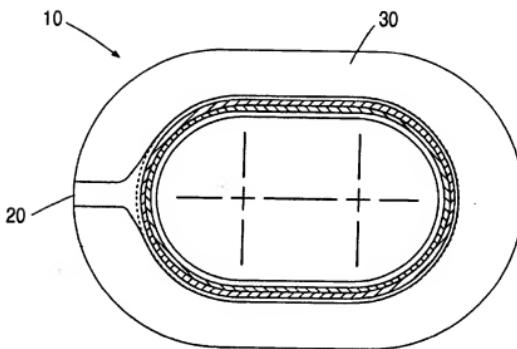


FIG. 2B

3/4

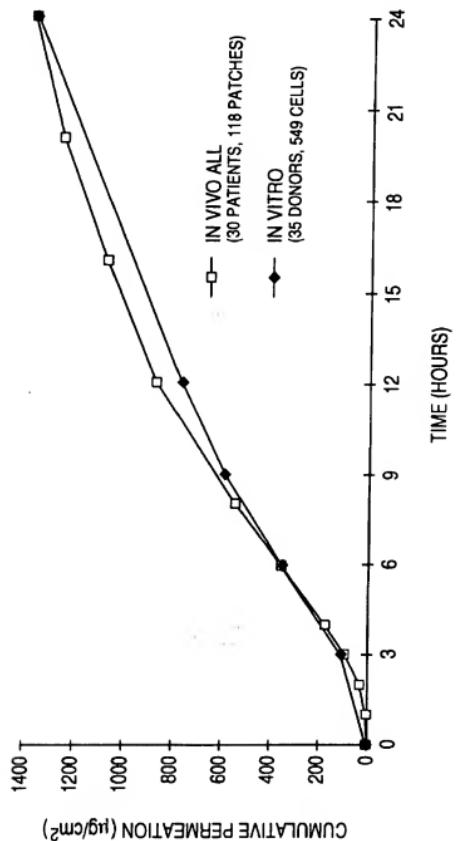


FIG. 3

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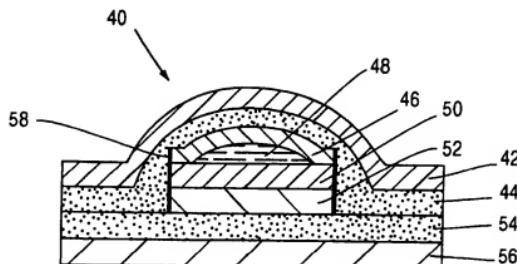


FIG. 4

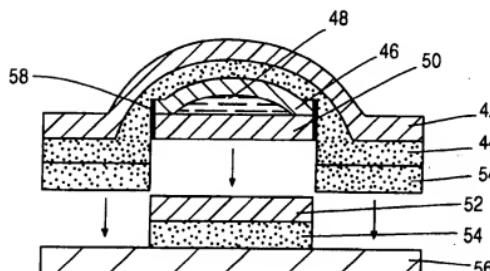


FIG. 5

INTERNATIONAL SEARCH REPORT

| | |
|------------------------------|-----------------|
| International application No | PCT/US 95/16498 |
|------------------------------|-----------------|

| |
|-------------------------------------|
| A. CLASSIFICATION OF SUBJECT MATTER |
|-------------------------------------|

IPC 6 A61K9/70

According to International Patent Classification (IPC) or to both national classification and IPC

| |
|--------------------|
| B. FIELDS SEARCHED |
|--------------------|

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

| |
|--|
| C. DOCUMENTS CONSIDERED TO BE RELEVANT |
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| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
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| Y | GB,A,2 184 016 (JONERGIN INC.) 17 June 1987 see page 1, line 121 - page 2, line 84 see figure 1 & US,A,4 710 191 cited in the application --- | 1-12 |
| A | EP,A,0 040 861 (KEY PHARMACEUTICALS) 2 December 1981 see page 12, line 33 - page 14, line 2 see figure 2 --- | 1-12 |

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'B' earlier document but published on or after the international filing date
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*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

*'&' document member of the same patent family

Date of the actual completion of the international search

8 May 1996

Date of mailing of the international search report

15.05.96

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Authorized officer

Benz, K

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/16498

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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International Application No.
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